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SUPPLEMENTAL FEEDING OF PREGNANT EWES[1]

S. B. WILLIAMS,[2] P. E. SYLVESTRE,[3] J. E. BOWSTEAD,[4] A. H. EWEN,[5] P. I. MYHR,[6] H. F. PETERS,[7]

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In the fall of 1946 a co-operative experiment on sheep nutrition was organized by and under the sponsorship of the Project Group of the Sub-Committee on Nutrition of the National Sheep Committee. Institutions participating in this work were the Central Experimental Farm at Ottawa, Ontario; Dominion Experimental Station at Swift Current, Saskatchewan; Dominion Range Experiment Station, Manyberries, Alberta; and the Departments of Animal Husbandry at the University of Alberta, Edmonton, Alberta, and the University of Saskatchewan, Saskatoon, Saskatchewan. The project leader was P. E. Sylvestre.

The ewes were subject to the experimental treatment for the winter feeding period of 1946-1947 and the trial was repeated with some modifications and standardization of procedure during the season of 1947-1948. The results presented cover both of these periods.

REVIEW OF LITERATURE

One of the more important problems in sheep husbandry is that of overwintering the pregnant ewe on a ration which will combine economy with maximum productivity and which will ensure that the subsequent lamb crop is not adversely affected as measured by size, development and vigour. There is considerable evidence that the plane of nutrition of pregnant ewes does affect the size and vigour of their offspring and has an influence on the subsequent growth of their lambs. Hammond (2) found that in a flock in which the individual ewes were graded and classified as to condition at lambing, those ewes which carried the most flesh gave birth to heavier lambs than did those that were less well fleshed. Thompson and Fraser (12) reported heavier birth weights for both single and twin lambs when ewes were well fed throughout gestation and in addition found that equal results as measured by birth weights were obtained when the poor ration was replaced by a liberal diet one month before lambing. They also reported a relationship between lamb birth weights and vigour—the smaller, lighter lambs being weaker. Underwood and Shier (13) gave one-half pound of wheat per head per day for the last 4 to 6 weeks of

[1] Contribution from the Project Group of the Sub-Committee on Nutrition of the National Sheep Committee, National Advisory Committee on Agricultural Services.

[2] Agricultural Scientist, Animal Husbandry Division, Central Experimental Farm, Ottawa, Canada, who prepared this report.

[3] Agricultural Scientist, Animal Husbandry Division, Central Experimental Farm, Ottawa, Canada.

[4] Associate Professor of Animal Hus andry, University of Alberta, Edmonton, Alberta.

[5] Professor of Animal Husbandry, University of Saskatchewan, Saskatoon, Saskatchewan.

[6] Animal Husbandry Division, Dominion Experiment Station, Swift Current, Saskatchewan.

[7] Officer-in-Charge, Dominion Range Experiment Station, Manyberries, Alberta.

pregnancy to a lot of ewes on grazing; a comparable check lot received no supplemental feeding. They found no difference in lamb birth weights under the two treatments but the small amount of supplement reduced the incidence of pregnancy toxaemia and lamb mortality. However, lambs that were heavier at birth grew faster; an increment of one pound in birth weight brought about a reduction of seven days in time required to reach a weight of between 65 and 70 pounds. Further work by these investigators (14), with higher levels of nutrition during the later stages of pregnancy, showed highly significant differences in birth weights in favour of the heavier rate of feeding. Subsequent lamb growth was better for the fed lot but this increased growth rate could be wholly accounted for by the increase in birth weight. They concluded that under their conditions very high levels of prenatal feeding are unlikely to be economic since the increase in growth rate from such treatment is not, on the present evidence, very great and losses of lambs and ewes can be prevented equally as well by a small supplement of grain. Wallace (15) (16) reported that the prenatal plane of nutrition markedly affects birth weights. He also reported a high correlation (0.7276) between birth weight and live weight increase to 28 days and that lamb vigour and viability were influenced profoundly by the plane of nutrition of their dams.

Since in many areas of this country legume hay cannot be grown, or if it is grown it is usually reserved for the dairy herd, it was proposed to determine the best method of utilizing roughage for pregnant ewes. Trials conducted at the Central Experimental Farm, Ottawa (11) have shown that timothy unsupplemented is not a suitable feed for pregnant ewes in that there is a heavy loss in body weight of the ewes, a high percentage of still-born lambs, a low percentage of good lambs, and a high subsequent lamb mortality.

Various workers have shown that the vitamin A content of timothy hay is lower than that of legume hay (6), (17) and it has been demonstrated that ample vitamin A must be provided for normal reproduction and normal health of the new born (3), (4), (5), (10). It was therefore decided to have three lots under test—one on legume hay, one on non-legume hay and one on non-legume hay changed to legume hay plus grain at mid-pregnancy. In addition, half of each of these lots would receive a vitamin A supplement.

PROCEDURE

An experimental outline was drawn up and was used by each Station. While in certain cases there was some variation between the co-operators these cases will be noted throughout the report as they arise. In general the procedure was as follows: A flock of ewes of the same or similar breeding was selected; these ewes were all bred to one ram or, if two rams were used, they were of similar breeding. During the breeding season all ewes received the same treatment with regard to feeding and management. Ochre was used on the rams and as soon as the ewes were judged to be in lamb they were allotted to three lots on the basis of breed, age, weight, condition and ram to which they had been bred. The treatment of the lots was then as follows:

Lot I—Legume hay plus mineral mixture.

Lot II—Non-legume hay plus mineral mixture.

Lot III—Non-legume hay plus mineral mixture from time of allotment until 100 days after the time the rams were put with the ewes; then legume hay plus mineral mixture plus a supplementary allowance of grain in the amount necessary to cause a steady gain in the live weight and condition of the ewes.

In addition, half of each lot received a weekly allowance of 50,000 I.U. of vitamin A. This was administered orally in capsule form and consisted of fortified high potency feeding oil. Roughage and minerals were fed ad libitum. All feeds were fed from racks or troughs.

The mineral supplement consisted of the following mixture:

50 pounds feeding bonemeal
50 pounds salt
0.8 ounces cobalt sulphate
0.5 ounces manganese sulphate
0.3 ounces copper sulphate
4.0 ounces ferrous sulphate
2.4 ounces stabilized potassium iodide.

Animals of each lot were penned together and bedded with straw. Access was given to a yard for exercise. Water was provided at least twice daily.

Lamb Birth Grades. All lambs were weighed and graded within 24 hours of birth. At the same time the sex and birth state (i.e. twin, single) of the lamb was recorded. The following grade standards were used:

(a) Good—Lambs of good weight, strong and which appear to have excellent chances of survival. Lambs in this category do not require any assistance at nursing.

(b) Fair—Lambs of good weight and fairly strong. These lambs should appear to have a good chance of survival. They may exhibit a little softness or sleepiness as opposed to brightness or life. They may require assistance at nursing for the first day.

(c) Weak—Soft, weak or small lambs. These lambs require assistance at nursing for a considerable period or may need extra heat and bottle feeding. Their chances of survival would appear to be negligible without the extra assistance. Whenever possible the cause of this grading was recorded.

(d) Dead—All lambs born dead or which die within a few hours of birth from causes other than accident. All defects and any evidences of abnormality or disease in the lambs or ewes were recorded.

Numbers, Breeds Used and Rations Fed

For convenience in preparing the tabular data all co-operators have been allotted a code letter and each institution will be identified in all subsequent tables by its code letter.

Institution	Code letter
Dominion Experimental Station, Swift Current, Sask.	A
Dominion Range Experiment Station, Manyberries, Alta.	B
University of Saskatchewan, Saskatoon, Sask.	C
University of Alberta, Edmonton, Alberta	D
Central Experimental Farm, Ottawa, Ontario	E

It will be noted that no data are presented for Station A for the season of 1947-1948 as this Station was unable to conduct the trial during that period.

In all, a total of 396 ewes were involved in the trial, 204 head in 1946-1947 and 192 head in 1947-1948. The distribution of these animals is shown in Table 1.

TABLE 1.—NUMBER OF EWES PER GROUP AT EACH STATION

Station	Lot I		Lot II		Lot III	
	Vit. A	No vit.	Vit. A	No vit.	Vit. A	No vit.
1946-1947						
A	5	5	5	5	5	5
B	5	5	5	5	5	5
C	7	7	7	7	7	7
D	8	8	8	8	8	8
E	9	9	9	9	9	9
Total 1946-1947	34	34	34	34	34	34
1947-1948						
B	8	8	8	8	8	8
C	8	8	8	8	8	8
D	7	7	7	7	7	7
E	9	9	9	9	9	9
Total 1947-1948	32	32	32	32	32	32
Total of both years	66	66	66	66	66	66

Over the two years there were available for comparison of the vitamin A treatment versus no vitamin A within each lot, 66 head; for the comparison of the various rations, 112 head; and for the comparison of the value of vitamin A over the three lots, 198 head.

The breeds of sheep used at each Station were as follows:

1946-1947

A—Rambouillet ewes bred to Romeldale ram
B—Romnelet ewes bred to Romnelet ram
C—Rambouillet ewes bred to Rambouillet ram
D—Suffolk, Hampshire and Romnelet ewes in the proportion of 6, 4, 6 bred to rams of their respective breeds
E—Grade Shropshire ewes bred to Shropshire ram.

1947-1948

B—Romnelet ewes bred to Romnelet ram
C—Rambouillet ewes bred to Rambouillet ram
D—Suffolk ewes bred to Suffolk ram
E—Grade Shropshire ewes bred to Shropshire ram.

The composition of the rations fed along with the amounts fed per head per day at the various Stations is shown in Tables 2A and 2B. At the foot of each table are notes indicating in a general way the type and quality of the various feeds.

While not all co-operators were able to obtain a chemical analysis of the feeds used during the first year of this trial, in the second year more complete data were obtained. Table 3 gives these figures and it will be noted that while the roughages fed at the various Stations differed quite widely in type, the difference as shown by these figures between the legume and non-legume hays is, with the exception of one Station, fairly constant.

As might be expected from the widely divergent geographical location of the co-operators, the dates of starting the trial, dates of lambing, etc., showed quite a wide range. As there was a change in procedure between the two years, these dates are reported in two tables, 4A and 4B. In the first year of the experiment several co-operators discontinued the feeding

TABLE 2A.—COMPOSITION OF RATIONS AND AMOUNT FED PER HEAD PER DAY, 1946-1947

Station	Lot I	Lot II	Lot III
A. 1st period	Alfalfa hay, 4.1 lb.	Oat sheaves, 4.3 lb.	Oat sheaves, 3.8 lb.
2nd period	Alfalfa hay, 4.1 lb.	Oat sheaves, 4.3 lb.	Alfalfa hay, 4.7 lb. Oats, 0.75 lb.
B. 1st period	Alfalfa hay, 5–8 lb.	Timothy $\frac{1}{4}$, Crested wheat grass $\frac{3}{4}$, 5–8 lb.	Timothy $\frac{1}{4}$, Crested wheat grass $\frac{3}{4}$, 5–8 lb.
2nd period	Alfalfa hay, 5–8 lb.	Timothy $\frac{1}{4}$, Crested wheat grass $\frac{3}{4}$, 5–8 lb.	Alfalfa hay, 5 lb. Oats $\frac{3}{4}$, Oilcake $\frac{1}{4}$, 1.0–1.5 lb.
C. 1st period	Alfalfa hay, 4 lb.	Oat sheaves, 4 lb.	Oat sheaves, 4 lb.
2nd period	Alfalfa hay, 4 lb.	Oat sheaves, 4 lb.	Alfalfa hay, 3.25 lb. Oats, 0.75 lb.
D. 1st period	Alfalfa hay, 6.25 lb.	Oat and brome hay, 6.25 lb.	Oat and brome hay, 6.25 lb.
2nd period	Alfalfa hay, 6.25 lb.	Oat and brome hay, 6.25 lb.	Alfalfa hay, 6.25 lb. Oats, 0.5 lb.
E. 1st period	Clover hay, 2.7 lb.	Timothy hay, 2.7 lb.	Timothy hay, 2.7 lb. Clover hay, 2.7 lb.
2nd period	Clover hay, 2.7 lb.	Timothy hay, 2.7 lb.	Oats $\frac{2}{5}$, bran $\frac{2}{5}$, Malt sprouts $\frac{1}{5}$–0.5 to 1.1 lb.

Notes regarding quality:

A. Alfalfa hay—good quality
Oat sheaves—good quality, contained considerable grain. Oat sheaves were put through a hammer mill

B. Alfalfa hay—good quality
Non-legume hay—good quality

C. Alfalfa hay—good quality
Oat sheaves—fair quality

D. Alfalfa hay—first cut was fed for first 50 days; then second cut containing about 25 per cent timothy was fed
Brome hay—good quality

E. Clover hay—fair quality. From April 8 on, second cut alfalfa hay of good quality was fed in place of clover hay
Timothy hay—good quality, bright and green.

TABLE 2B.—COMPOSITION OF RATIONS AND AMOUNT FED PER HEAD PER DAY, 1947-1948

Station	Lot I	Lot II	Lot III
B. 1st period	Alfalfa hay, 4 lb.	Crested wheat grass hay, 3.9 lb.	Crested wheat grass hay, 3.9 lb.
2nd period	Alfalfa hay, 4 lb.	Crested wheat grass hay, 4.0 lb.	Alfalfa hay, 4.0 lb. Oats, 0.93 lb.
C. 1st period	Alfalfa hay, 4 lb.	Oat sheaves and Meadow hay, 5.0 lb.	Oat sheaves and Meadow hay, 5.0 lb.
2nd period	Alfalfa hay, 4 lb.	Oat sheaves and Meadow hay, 5.0 lb.	Alfalfa hay, 4.0 lb. Wheat screenings, 0.5 lb.
D. 1st period	Alfalfa and Timothy hay, 5.21 lb.	Brome hay, 4.86 lb.	Brome hay, 4.90 lb.
2nd period	Alfalfa and Timothy hay, 5.29 lb.	Brome hay, 5.03 lb.	Alfalfa and Timothy hay, 4.64 lb., Oats, 0.5 lb.
E. 1st period	Clover and Alfalfa hay, 2.98 lb.	Timothy, 3.01 lb.	Timothy, 3.00 lb.
2nd period	Clover and Alfalfa hay, 3.11 lb.	Timothy, 3.42 lb.	Clover and Alfalfa hay, 3.09 lb. Oats, 0.92 lb.

Notes regarding feed quality:

B. Alfalfa hay—first cut, sun-cured and baled
Crested wheat grass hay—sun-cured and loosely stacked
90 per cent crested wheat grass, 10 per cent wild barley, spear grass and sweet clover

C. Alfalfa hay—fair quality
Oat sheaves—good quality
Meadow hay—fair quality

D. Alfalfa and timothy hay—fair quality, 50 per cent alfalfa
Brome hay—good quality
Oats—feed oats weighing about 38 pounds per bushel

E. Clover and alfalfa hay—fair quality mixed clover and alfalfa with about 25 per cent timothy, field baled in round bales
Timothy hay—fair quality with from 10 to 20 per cent clover
Oats—good quality feed oats.

of the experimental rations as soon as the first ewe lambed, while in 1947-48 all ewes were kept on the experimental ration until they had lambed with the exception of those of co-operator "C", these sheep being removed from the trial at the end of March. Lambing in this group started on April 12. In all lots the date of change-over in the ration of Lot III was also the date on which the intermediate weighing of the ewes took place. Thus Stations showing the longest period from start to date of change-over could also be expected to show the greatest differences in ewe body weight at this time. In Tables 4A and 4B "Init." refers to the date on which the ewes were started on the experimental rations and "Inter." is that on which the ration of Lot III was changed to legume hay plus grain. In Table 4A the last columns refer to the actual number of days to first lambing while the same columns in Table 4B contain the average number of days to the actual lambing date of each ewe.

TABLE 3.—CHEMICAL COMPOSITION OF FEEDS IN PER CENT

Station	Feed	Protein	Ether extract	Crude fibre	Ash	N.F.E.
1946-1947						
A.	Alfalfa hay	16.1	—	32.0	—	—
	Oat sheaves	9.7	—	32.4	—	—
E.	Clover hay	13.3	0.9	35.5	6.4	43.9
	Timothy hay	8.5	0.9	43.7	5.7	41.1
	Grain mixture	16.7	4.0	13.2	4.9	61.2
1947-1948						
B.	Alfalfa hay	14.1	0.6	44.6	6.2	34.6
	Crested wheat hay	9.9	1.3	31.4	8.3	49.2
	Oats	16.1	4.4	13.0	3.6	62.8
C.	Alfalfa	14.6	2.1	27.1	8.6	—
	Oat sheaves	8.4	3.7	22.4	4.6	—
	Meadow hay	5.7	2.1	31.2	5.6	—
	Wheat screenings	15.5	2.8	3.6	2.3	—
D.	Alfalfa and Timothy hay	13.0	2.1	36.4	6.5	36.6
	Brome hay	12.2	2.0	28.4	5.0	48.0
	Oats	12.7	6.2	11.5	3.3	61.3
E.	Clover and Alfalfa hay	10.7	0.9	42.3	5.7	40.4
	Timothy hay	7.2	1.3	39.9	5.3	46.2
	Oats	14.6	4.9	13.0	3.3	64.1

TABLE 4A.—LENGTH OF PERIODS, 1946-1947

Station	No. days Init. to Inter.	No. days Inter. to Lamb.	No. days Init. to Lamb.
A	61	40	101
B	34	77	111
C	33	52	85
D	77	68	145
E	53	40	93
Average	52	55	107

TABLE 4B.—LENGTH OF PERIODS, 1947-1948

Station	No. days Init. to Inter.	Av. no. days Inter. to Lamb.	Av. no. days Init. to Lamb.
B	56	56	112
*C	100	37	137
D	63	57	120
E	54	68	122
Average	68	54	123

* "Init." was the start of the breeding season and "Lamb." was the date when the ewes were removed from e experimental ration.

EXPERIMENTAL RESULTS

Although the original outline of the trial did not call for post-natal mortality records, as there were indications that there might be differences in mortality rates, particularly between vitamin A and no vitamin A treatment, all Stations were asked to submit their mortality figures. Data were obtained from all but one Station and are summarized in Table 5.

TABLE 5.—LAMB MORTALITIES 1947 AND 1948

	Lot I		Lot II		Lot III		All lots	
	Vit. A	No A	Vit. A	No A	Vit. A	No A	Vit. A	No A
Live births	85	81	82	74	82	83	249	238
Mortality:								
To 28 days	13	18	15	15	14	17	42	50
To weaning	18	26	21	25	17	20	56	71
Per cent mortality:								
To 28 days	15	22	18	20	17	20	17	21
To weaning	21	32	26	34	21	24	22	30
Per cent mortality of lot:								
To 28 days	19		19		19		19	
To weaning	26		30		22		26	

While the differences in mortality rates seem consistent and in line with what might be expected, yet in no case do they approach significance. This is doubtless due to the tremendous range in percentages both between and within Stations and lots (0 to 73 per cent).

Table 6 presents the results for 1947 and 1948 as well as a summary of the two years.

DISCUSSION

Due to the nature of the data and the widely different feeds and environmental conditions to which the lots were subjected at the different locations, a statistical analysis of the data has not been carried out. The following points, however, were common to all Stations and justify certain conclusions:

(a) At each Station there were three lots; one received a legume hay, one received a non-legume hay and the third received non-legume hay until approximately mid-pregnancy and from then on was given legume hay plus a grain supplement.

(b) At each Station half of each lot received a high potency vitamin A supplement.

(c) All sheep were allowed free access to a mineral supplement.

The following observations are made:

(a) Inspection of Table 6 shows that legume hay plus minerals gave more satisfactory results when fed to pregnant ewes than did non-legume hay. Ewes fed legume hay maintained their weight more satisfactorily and gave birth to heavier, more vigorous lambs. Although there is a

TABLE 6.—SUPPLEMENTARY FEEDING OF PREGNANT EWES. ALL STATIONS. SUMMARY 1947 AND 1948

	Lot I Legume			Lot II Non-Legume			Lot III Changeover			All	
	Vit. A	No Vit. A	All	Vit. A	No Vit. A	All	Vit. A	No Vit. A	All	Vit. A	No Vit. A
1947											
No. ewes	34	34	68	34	34	68	34	34	68	102	102
Av. gain Init.—Lamb.	−6.7	−4.6	−6.5	−11.2	−11.5	−11.4	−7.9	−6.6	−7.3	−8.5	−7.6
No. ewes died	0	1	1	3	1	4	0	0	0	3	2
No. ewes lambed	31	27	58	28	26	54	28	30	58	87	83
No. live births	54	44	98	39	36	75	40	44	84	133	124
No. dead births	3	1	4	3	3	6	1	0	1	7	4
Per cent "Good" lambs	74	67	71	64	56	60	80	93	87	73	73
Av. birth weight	8.1	8.1	8.1	8.8	8.1	8.5	8.7	9.1	8.9	8.6	8.4
Lambing per cent	174	163	169	139	138	139	143	147	145	153	149
1948											
No. ewes	31	32	63	32	32	64	32	32	64	95	96
Av. gain Init.—Lamb.	−1.8	−4.5	−3.2	−18.3	−13.0	−15.6	−3.6	−3.5	−3.5	−8.1	−7.0
No. ewes died	1	1	2	0	1	1	0	0	0	1	2
No. ewes lambed	28	30	58	31	31	62	30	31	61	89	92
No. live births	41	43	84	51	43	94	47	45	92	139	131
No. dead births	1	2	3	2	3	5	2	1	3	5	6
Per cent "Good" lambs	74	69	71	58	56	58	77	65	72	69	63
Av. birth weight	8.2	8.3	8.3	7.3	7.5	7.4	8.5	8.5	8.5	8.0	8.1
Lambing per cent	146	143	145	164	139	152	157	145	151	156	142
1947 and 1948											
No. ewes	65	66	131	66	66	132	66	66	132	197	198
Av. gain Init.—Lamb.	−4.4	−4.6	−4.5	−14.6	−12.2	−13.4	−5.8	−5.1	−5.5	−8.3	−7.3
No. ewes died	1	2	3	3	2	5	0	0	0	4	4
No. ewes lambed	59	57	116	59	57	116	58	61	119	176	175
No. live births	95	87	182	90	79	169	87	89	176	272	255
No. dead births	4	3	7	5	6	11	3	1	4	12	10
Per cent "Good" lambs	74	68	71	61	56	59	79	79	79	71	68
Av. birth weight	8.1	8.2	8.2	8.0	7.8	7.9	8.6	8.8	8.7	8.2	8.3
Lambing per cent	161	153	157	153	139	146	150	146	148	155	146

difference in lambing percentage in favour of the lot fed legume hay, assuming a coefficient of variability in lambing percentage of 35 per cent, this difference is less than is required for significance. This is to be expected since the ewes were not—with the exceptions previously noted—put on the experimental rations until after breeding and normally eggs shed by the ovulating ewe develop successfully unless they are not, as sometimes happens, fertilized or implanted (8). Thus the treatments should not have affected the lambing percentages.

(b) Under the conditions of the experiment the addition of a vitamin A supplement to any of the rations did not improve the ration as measured by maintenance of ewe body weight, or birth weight and vigour of the lambs. While once again there were differences in mortality rates and lambing percentages between the lots, these can be explained on the basis of normal variability. This is in agreement with the findings of Hart and Miller (4) who reported that reproduction was not impaired in sheep unless the ewes were vitamin A-depleted before the start of a winter feeding period trial. While carotene analyses were obtained from only one Station (E), it is of interest to note that these show that even the ewes on the non-legume roughage were receiving two to three times their normal requirements (1), (7), (9) of this vitamin.

SUMMARY

During the winter of 1946-47 five institutions co-operated in conducting an experiment with pregnant ewes under a common project outline. At each institution the following lots were fed: *(a)* legume hay throughout pregnancy; *(b)* non-legume hay throughout pregnancy; *(c)* non-legume hay until mid-pregnancy, then legume hay plus grain. Half of each lot received a high potency vitamin A supplement. The trial was repeated the following winter. Under the conditions of the experiment:

(a) Legume hay gave more satisfactory results than did non-legume hay as measured by maintenance of ewe body weight throughout pregnancy, lamb birth weight and lamb vigour at birth.

(b) The change-over ration gave results comparable to those obtained when legume hay alone was fed.

(c) The addition of a vitamin A supplement to these rations did not bring about any significant improvement.

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CORRELATION STUDIES OF YIELD AND OTHER CHARACTERS IN RYE POLYCROSSES[1]

F. S. Warren and H. K. Hayes[2]

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Although important in the economy of the United States, rye has not generally been subjected to an intensive improvement program. The desirability of such a program is indicated by the uniformity of the average yields during a period when the average yields of other crops, notably corn and wheat, have been rising steadily. Also worthy of note is the rarity of introduction of new improved rye varieties.

Several reasons account for the lack of emphasis on rye breeding. The mode of pollination presents some special problems. Rye is cross-pollinated so that breeding by selfing, selection and crossing should be effective. However, self-sterility complicates the process of inbreeding and the rather high proportion of unfruitful florets after natural cross-pollination offers another difficulty.

Present world demands for food and feed, however, are so insistent that investigation of the possibilities presented by new breeding methods applicable to rye is of considerable interest. One such breeding method, being studied at the University of Minnesota, is the use of the polycross for testing the performance of rye inbreds and for the subsequent combining of selected progenies.

The methods used in these studies consisted of the selection of plants that had a relatively high seed-setting when self-pollinated. Selection was made also for desirable seed and plant characters. The selected lines were combined in two polycross nurseries, one containing lines relatively homozygous for seeds with colourless aleurone and the other, lines homozygous for green aleurone. The performance in polycross yield trials was studied in relation to the length of time that the lines had been self-pollinated and to the extent of self-fertility of the inbreds. A major purpose of this investigation was to make an intensive study of the characters of the polycrosses in comparison with standard varieties. On the basis of these results various combinations of inbreds were selected for the production of synthetic varieties.

REVIEW OF LITERATURE

Earlier investigations with rye are of interest in relation to this study. Previous reports by Brewbaker (1) and Peterson (4) in Minnesota have shown that self-fruitful plants can be obtained sufficiently frequently to make selection in self-pollinated lines possible for seed and plant characters and to produce seed in sufficient quantity for polycross studies. Peterson's results indicate that self-fertility vs. self-sterility is not a simply inherited character in rye; frequently, in the early generations of selfing, both self-fertile and self-sterile plants are obtained in the progeny of a self-

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[2] Graduate Student, and Professor and Chief, respectively, University of Minnesota.

pollinated plant that itself was high in seed setting under conditions of self-pollination. Leith and Shands (3) also have studied the effects of long continued selfing. They, like Brewbaker, suggested compositing selfed lines to produce synthetic varieties. A variety recommended by the Minnesota station, named Emerald, was produced in this manner. Krasniuk (2) reports a rye inbred line that after six generations of selfing yielded 46 per cent more than the local rye variety.

The polycross method of determining combining ability in alfalfa was originally described by Tysdal, Kiesselbach and Westover (6). The selected clones were grown together in a single nursery and so randomized that outcrosses of these would be effected by a reasonably comparable sample of pollen. Seed was bulked from all replicates of the same clonal line and used in a performance trial. Better combiners, as indicated by their performance, were selected to produce a synthetic. Other advantages of the method were stated to be (*a*) ample seed produced for testing; (*b*) self-sterile lines could be tested effectively; (*c*) inbreeding was not required.

Tysdal and Crandall (5) later compared the polycross method with top cross and average single cross performance of the same eight clonal lines as a means of selecting high combining clones in alfalfa. For the four characters studied, including forage yield, cold resistance, bacterial wilt resistance, and leaf hopper resistance, the eight clones ranked practically the same by each method. The synthetic variety resulting from recombining clones selected on the basis of polycross performance was significantly higher in yield than the standard varieties or the synthetic composed of low combining clones selected by the same method.

MATERIALS AND METHODS

The selfed lines selected to be grown in the polycross crossing plots were those that had medium-to-high seed production under conditions of self-pollination, when several heads of a plant were isolated in vegetable parchment bags. Only the more vigorous selfed plants and lines were selected. Selection was only on a plant basis for the lines that originated from plants of Dakold and Imperial that had been selfed only for the first time. Selfed plants were selected for the polycross crossing plots that excelled in seed size, plumpness and quality of seed, and that appeared to be breeding relatively true for either colourless or green seed colour.

Each polycross crossing plot was made up of individual plant hills six inches apart, in rows one foot apart, arranged in a randomized block of thirty replications. The thirty hills of each inbred were thus associated in a random manner with the other inbreds in the nursery. Every plant should have been pollinated with a random selection of pollen from other lines in the nursery except for differences resulting from variations in time of shedding pollen, differential viability, cross sterility, and other similar causes. The individual plants of each line were harvested together, threshed and the seed bulked for seeding in the performance trials. Originally, there were 64 colourless aleurone lines in Polycross Nursery I and 23 green aleurone lines in Polycross Nursery II. The yield trials and study of characters of polycrosses were made from 30 colourless lines and 13 green aleurone lines. These are the lines that appeared relatively homozygous

TABLE 1.—ORIGIN, YEARS SELFED, CLASSIFICATION FOR SEED COLOUR AND FOR SELF-FERTILITY OF SELFED LINES OF RYE USED IN POLYCROSS YIELD TRIALS

Polycross line No.	Seed colour	Origin	Years selfed	Fertility group
2	Colourless	(High × Low) × Minn. 111	5	1
3	Colourless	High fertility	19	1
4	Colourless	Sagnitz	16	2
6	Colourless	Dakold	8	1
8	Colourless	Dakold	5	1
10	Colourless	Dakold	4	2
12	Colourless	Dakold	3	2
17	Colourless	Dakold	3	3
21	Colourless	Dakold	1	4
22	Colourless	Dakold	1	4
24	Colourless	Dakold	1	3
25	Colourless	Dakold	1	2
29	Colourless	Dakold	1	5
30	Colourless	Dakold	1	2
31	Colourless	Dakold	1	4
34	Colourless	Dakold	1	4
35	Colourless	Dakold	1	4
37	Colourless	Dakold	1	4
41	Colourless	Dakold	1	4
48	Colourless	Imperial	1	5
50	Colourless	Imperial	1	3
51	Colourless	Imperial	1	3
52	Colourless	Imperial	1	3
53	Colourless	Imperial	1	4
55	Colourless	Imperial	1	3
56	Colourless	Imperial	1	3
58	Colourless	Imperial	1	4
59	Colourless	Imperial	1	4
61	Colourless	Imperial	1	3
64	Colourless	Imperial	1	4
13	Green	Dakold	3	3
65	Green	Dakold	5	2
66	Green	Dakold	8	2
67	Green	Dakold	5	2
71	Green	Dakold	3	5
72	Green	Dakold	2	5
73	Green	Dakold	2	2
74	Green	Dakold	1	5
75	Green	Dakold	1	5
76	Green	Dakold	1	3
77	Green	Dakold	1	5
78	Green	Dakold	1	3
81	Green	Dakold	1	3

for seed colour on rechecking, that appeared most desirable as inbreds, that produced sufficient seed for the yield trials and for which sufficient selfed seed was available to continue the line.

The inbred lines grown in the polycross yield trials are listed in Table 1. The origin, generations selfed when first grown in the polycross nurseries and classification of lines for self-fertility are given.

Classification for self-fertility was based on 1947 data for lines listed as selfed only once, while lines selfed for a longer period were studied in both the 1946 and 1947 crop seasons. They were placed in five self-fertility groups as follows: (1) all plants highly self-fertile; (2) nearly all plants highly self-fertile with an occasional self-unfruitful plant; (3) approximately

one-half of plants highly self-fertile; (4) approximately one-fourth plants highly self-fertile; (5) all plants medium-to-low in self-fertility. In group 5 it was necessary to combine seed from several plants.

The performance of the polycrosses was tested in two groups arranged to include nearly equal numbers of lines. Group I was made up of twenty-one lines of which nine were obtained from the colourless selections and twelve from the green selections in the polycross plots. Group II was made up of twenty-two lines from the colourless selections. The individual lines, selected to be grown in the polycross yield trials, were those that had no extremely undesirable plant characters in the polycross crossing plots. They also produced sufficient seed for the yield trials and produced grain of comparatively good quality, size and plumpness. The three standard varieties recommended for Minnesota—Dakold, Acc. No. 93, Imperial, Acc. No. 115, and Emerald, Acc. No. 93—were included in each group.

Each polycross line and variety was planted, when sufficient seed was available, in single rod-row plots replicated six times. In Group I, five lines were replicated only three times and two others were replicated four times because of insufficient seed. Rate of seeding was five hundred seeds per eighteen-foot row, and the rows were spaced one foot apart. The two groups of polycrosses were planted in adjacent areas on September 13, 1946. Replications for Polycross Group I were sown in rows 1 to 128, inclusive and those for Polycross Group II in rows 129 to 278, inclusive.

Planting conditions were good and first emergence was noted on September 17. The stand of most of the polycross progenies was equal to that of the standard varieties. Fall growth vigour ratings were made on October 21, 1946, on the basis of the appearance of the rows. Classes of one to five were used with one representing poor vigour and five very good vigour. At this time, most plants appeared in good condition to survive the winter.

Winterkilling was negligible, and in the spring most lines made rapid early growth. Stooling vigour was rated in the first week of May in classes of one to five. A rating of five represented very good stooling vigour, while a rating of one indicated poor stooling vigour.

Plants of many of the polycross progenies began to head out during the latter part of May. A row was recorded as headed when approximately fifty per cent of the plants in the row had produced heads. The majority of the polycrosses were in head by the end of the first week of June. Height at maturity was measured as the average for the row and the uniformity of height was also estimated. Since all rows were classified as eighty per cent or better in uniformity, only the average height for each row was recorded.

At maturity, a one-foot border of plants was cut off each end of every row and discarded. Each plot, consisting of a 16-foot row, was harvested and threshed separately. Yields in grams of individual rows were converted to yields in bushels per acre by the use of a factor.

During the harvesting operation, a sample of twenty-five normal heads was collected at random from each row and placed in an envelope for later use in the head and kernel studies. The total number of lateral florets, exclusive of those in the lowermost and terminal spikelets which rarely produce seeds, was counted for each of ten heads from each row. The

seeds from the ten-head sample were then rubbed out, counted, and the total number of seeds produced was expressed as a percentage of the total number of florets for per cent fruitfulness. The weight in grams of this known number of kernels was determined and the weight per hundred kernels was calculated.

Studies were made on pollen sterility of the polycrosses during two seasons. The per cent of quartets with micronuclei during microsporogenesis was also studied. For each pollen sample an entire head was taken, labelled and stored in 70 per cent alcohol for subsequent pollen sterility determinations. Sterility was estimated in the following manner. A whole anther was removed from a floret near the region where the anthers were extruded and its entire contents squeezed into a strong potassium iodine solution. Pollen grains of normal size and more than one-half filled with starch were considered normal and all others as abortive. The per cent sterility was estimated with reference to specially prepared slides showing various degrees of sterility verified by counting large numbers of pollen grains. Pollen sterility was estimated on at least two separate occasions for each sample, the average of these estimations being used in the analysis.

Sporocyte material was collected and placed in vials containing freshly prepared killer solution made up of three parts of 95 per cent alcohol and one part of glacial acetic acid. The samples were immediately stored in a refrigerator at about 36° F. After thirty-six hours, the killer solution was replaced by 70 per cent alcohol and the samples returned to the refrigerator.

Microsporogenesis was studied by means of the iron aceto-carmine smear technique. Smears were made of complete anthers in the various stages of meiosis in an attempt to locate irregularities of division which might lead to pollen or ovule sterility and, in turn, influence yield.

Particular emphasis was placed on the examination of spore quartets for micronuclei. The quartet stage was located and smears prepared from two anthers, wherever possible from different florets, of each plant. In each preparation, approximately two hundred quartets were classified according to the number of micronuclei present. The per cent of quartets with micronuclei was then calculated.

Analyses of variance to determine the significance of varietal differences were calculated for Group I and Group II separately for eight characters, namely, fall growth vigour; stooling vigour; date of heading; height of plant; weight per bushel; yield in bushels per acre; per cent fruitfulness, and weight of one hundred kernels. Per cent data were converted to degrees of $\sin^2 \theta$ before analysis. The significance of varietal differences in pollen sterility was determined by analysis of variance using data for the two years as replicates.

Simple product moment correlation coefficients were calculated to determine the degree of association between yield and the other agronomic characters. On the basis of these correlation coefficients, eight characters, namely, yield; fall growth vigour; stooling vigour; date of heading; weight per bushel; per cent pollen sterility; per cent fruitfulness, and weight per hundred kernels, were selected for further correlation studies.

Correlation coefficients were calculated for all interrelationships of these eight characters. Utilizing these simple product moment correlation coefficients, the partial correlation coefficients for all combinations of the eight characters were calculated. The multiple correlation for the dependence of yield on the other seven characters was then determined.

After learning the relative value of various seed and plant characters in relation to yielding ability, the data obtained were used in selecting inbred lines for the production of several synthetic varieties.

In reporting the statistical results of this study, a single asterick (*) is used to indicate significance at the five per cent level and a double asterisk (**) to indicate significance at the one per cent level.

EXPERIMENTAL RESULTS

Characters of Polycrosses in Relation to Three Standard Varieties

Agronomic Characters

There were twenty-one polycrosses and three varieties grown in the yield trials for Group I and twenty-two polycrosses and the three varieties in yield Group II. Separate analyses of variance were made for Groups 1 and 2, respectively. Differences between entries were highly significant in both groups for the following characters: fall growth vigour; stooling vigour; date of heading; height of plant; weight per bushel; per cent fruitfulness; and yield in bushels per acre. Differences between entries for weight of 100 kernels was highly significant in yield Group II and significant at the 5 per cent level in Group I.

As the three recommended varieties, Dakold, Emerald and Imperial, were grown in each of the two yield trials, they were used as a basis for the study of characters of the polycrosses grown in the same yield group. In Table 2 the frequency distribution of twenty-one polycrosses in Group I and twenty-two polycrosses in Group II is shown in relation to the mean of the three standard varieties grown with each group. Class centres of the distribution are separated by multiples of the least significant difference at the five per cent level, except for class 0, and are arranged in plus and minus classes on each side of the zero class. The zero class contains all means that did not differ from the average of the three varieties by as much as plus one or minus one times the least significant difference. Class + 1.5 contains all means whose value fell within the range of + 1 to + 1.9 times the least significant difference for that character. Similarly the other classes contain means within the appropriate range limits. In this way all polycrosses in classes other than the zero class differ significantly from the average of the means of the three standard varieties for that character.

Comparisons of the polycrosses with the standard varieties may be made from the frequency distributions. For both fall growth vigour and stooling vigour, the polycrosses were, in general, clearly superior to the means of the three varieties. For general combining ability, these two measures of vigour show that the polycrosses were outstanding when compared with the average of the varieties. This leads to the conclusion that most of the seed produced in the polycross crossing plots was a result of cross-pollination.

TABLE 2.—FREQUENCY DISTRIBUTION FOR AGRONOMIC CHARACTERS OF 21 POLYCROSSES IN GROUP I, AND 22 POLYCROSSES IN GROUP II. CLASS CENTRES ARE SEPARATED BY MULTIPLES OF THE L.S.D. ARRANGED ± ON EACH SIDE OF THE 0 CLASS WHICH IS REPRESENTED BY THE AVERAGE OF THE THREE VARIETIES IN RESPECTIVE GROUP

Character	Group	−3.5 or more	−2.5	−1.5	0	+1.5	+2.5	+3.5 or more	Total
Fall growth vigour	I	—	—	—	3	11	7	—	21
	II	—	—	—	—	—	2	20	22
Stooling vigour	I	—	—	—	2	3	6	10	21
	II	—	—	—	—	—	—	22	22
Date heading	I	—	3	8	10	—	—	—	21
	II	—	2	2	14	4	—	—	22
Height	I	—	—	6	14	1	—	—	21
	II	—	—	1	20	1	—	—	22
Weight per bushel	I	1	2	1	17	—	—	—	21
	II	—	2	6	14	—	—	—	22
Yield bu./acre	I	—	—	1	13	6	1	—	21
	II	—	—	—	7	13	2	—	22
Per cent fruitfulness	I	10	1	1	3	3	0	3	21
	II	11	2	0	3	2	1	3	22
Weight of 100 kernels	I	—	—	—	21	—	—	—	21
	II	—	—	3	19	—	—	—	22

Earliness, as indicated by date of heading, was exhibited by many of the polycrosses by comparison with the average performance of the standard varieties. A total of fifteen polycrosses headed out significantly earlier than the average of the varieties, while four polycrosses were significantly later. Twenty polycrosses matured significantly earlier and one significantly later than the average of the varieties.

The distribution of the polycrosses for height of plant was limited to a range of class centres 1.5 times the least significant difference on each side of the mean of the standard varieties. Altogether seven polycrosses were significantly shorter than the average of the varieties, while two were significantly taller.

The majority of the polycrosses did not differ significantly from the average of the varieties in weight per bushel. However, twelve had a significantly lower weight per bushel than the average of the varieties. Only one of the polycrosses had a significantly lower yield in bushels per acre than the mean of the varieties, while twenty-two were significantly superior in yield.

Comparisons may be made also in regard to the head and kernel characters studied. Per cent fruitfulness showed the widest range in variability of the characters studied. A total of twelve polycrosses significantly exceeded the average of the three varieties, while twenty-two had a significantly lower per cent of fruitfulness. Eighteen of those with a lower per cent of fruitfulness were more than four times the least significant

difference below the average of the varieties. Many of the lines used in these polycrosses, as has been stated, originated from the Dakold variety. It will be noted that Dakold was lower in fruitfulness than either Emerald or Imperial in both groups of yield trials. When the polycrosses are compared directly with Dakold, the majority are equal to the parent variety or superior.

For weight of one hundred kernels the range in distribution about the mean of the varieties was extremely limited. Only three polycrosses differed significantly from the mean of the three varieties and for each of these the weight was lower than that of the varieties.

Pollen Sterility

The sterility determinations for 1947-48 were made on plants grown from remnant seed available from the polycross crossing plot. Sterility ranged from a low of two per cent for polycross 72 in 1947-48 to a high of sixty per cent for polycross 65 during the same season. The majority of the polycrosses were below fifteen per cent in pollen sterility. In order to determine the consistency of the pollen sterility estimations for the two years, the degree of association between the two estimations for those polycrosses grown in both years was calculated. The correlation coefficient for this association was + 901** for thirty-one polycrosses and was significant at the one per cent level. Realizing that the high sterility of Polycross 65 contributed disproportionately to this high coefficient, the degree of association was recalculated eliminating this polycross. The resulting coefficient, + 611**, was highly significant. Such a strong association indicated that the pollen sterility of the polycross was fairly similar in these two years and that the method of estimation was reasonably reliable.

Quartet Analysis

Twenty of the polycrosses were studied for the per cent of quartets showing micronuclei. Pollen sterility was determined from pollen of the same plants. A total of thirty-two plants were examined in this study. The numbers of quartets examined for each of two anthers per plant that were studied ranged from 81 to 401.

The per cent of micronuclei ranged from a low of zero for six plants to a high of 17.6 for polycross number 25 plant 4. The average per cent of abnormal quartets for all the plants examined was 1.35, while the average pollen sterility of these plants was 19.8. The sterility of the plants within a polycross was extremely variable, as also was the per cent of abnormal quartets. However, the variability in per cent of micronuclei between different anthers of the same plant was negligible in every case.

The degree of association between per cent of pollen sterility and per cent of quartets with micronuclei was calculated by means of the simple product moment correlation formula. In order to overcome the mathematical difficulties involving the use of zero categories, the data for per cent of abnormal nuclei were transformed to per cent normal quartets by use of the reciprocal. Both sets of percentage data were transformed to degrees of $\sin^2 \theta$. The association between per cent sterility and per cent normal quartets was of no importance or significance as shown by the correlation coefficient of − 0.066.

This low degree of association indicates that chromosomal abnormalities and difficulties of division resulting in micronuclei in the spore quartets are not a major cause of pollen sterility in these polycrosses. Presumably the cause of the pollen sterility must lie in some physiologic process under genic control.

Correlation Between Yield and Other Characters

Correlations between yield and other characters were studied by using the data in yield Groups I and II together. Before making these calculations a comparison was made for each of the characters studied including fall growth vigour; stooling vigour; date of heading; weight per bushel; weight per 100 kernels; per cent fruitfulness and yield for the three varieties grown in the two groups of yield trials which were made in adjacent areas. The comparisons were made by the t test. Except for the two characters yield and per cent fruitfulness there were no significant differences between the two yield trials for the average of these three varieties.

For yield the average of the three varieties in yield Group I was 58.6 bushels and in Group II, 48.7 bushels. The difference was highly significant. Percentage yield of each of the polycrosses in each group, therefore, was calculated in terms of the average yield of the three varieties taken as 100 when grown in the same group.

As the chief difference in per cent fruitfulness was due to a rather wide difference in reaction of the Imperial variety it was not considered necessary or desirable to correct this character. In these correlations the 43 polycrosses were studied as a group.

Simple product moment correlation coefficients were calculated for all interrelationships of the eight characters, fall growth vigour; stooling vigour; date of heading; weight per bushel; per cent fruitfulness, yield and weight per 100 kernels (see Table 3).

Several of the correlation coefficients were significant at the one per cent level, though all r values were less than $\pm$ 0.5. Yield was highly significantly positively associated with stooling vigour and with per cent fruitfulness. As might be expected, profuse stooling and a high per cent

TABLE 3.—SIMPLE PRODUCT MOMENT CORRELATION COEFFICIENTS FOR ALL INTERRELATIONSHIPS OF EIGHT CHARACTERS OF THE 43 POLYCROSSES OF RYE IN GROUPS I AND II

Character	A Yield	B Fall growth vigour	C Stooling vigour	D Date of heading	E Weight per bu.	F Pollen sterility	G Per cent fruitfulness
B Fall growth vigour	+.366*						
C Stooling vigour	+.448**	+.313*					
D Date of heading	+.274	+.374*	+.139				
E Wt. per bu.	+.186	−.183	−.283	−.314*			
F Pollen sterility	−.373*	−.384*	−.446**	−.301*	+.402**		
G Per cent fruitfulness	+.422**	+.023	+.223	+.245	−.144	−.263	
H Wt. per 100 kernels	+.017	−.261	−.143	+.006	+.051	+.169	+458**
D.F.	41	301*	389**				

of fruitfulness generally result in increased yields. Yield was also positively associated with fall growth vigour and negatively associated with pollen sterility, both significantly at the five per cent level. The association of yield and date of heading approached significance in a positive direction. Other associations with yield were extremely weak.

Fall growth vigour showed some significant associations with other characters, though none was highly significant. Fall growth vigour and stooling vigour were significantly positively associated, as were fall growth vigour and date of heading. The association of fall growth vigour and pollen sterility was significant in a negative direction.

Stooling vigour was highly significantly negatively correlated with pollen sterility. Polycrosses with poor stooling vigour might, therefore, be undesirable also due to high pollen sterility. Two other associations of stooling vigour, while not significant, may be of interest. The association of stooling vigour with weight per bushel approached the significant level in a negative direction, while that with per cent fruitfulness was positive.

Date of heading was significantly negatively associated with both weight per bushel and pollen sterility at the five per cent level. Earlier polycrosses had a slight tendency to have higher weight per bushel and higher pollen sterility; however, many polycrosses could be expected to show a combination of the desirable characters. The association of date of heading and per cent fruitfulness approached significance in a positive direction.

TABLE 4.—SOME SIMPLE AND PARTIAL CORRELATION COEFFICIENTS OF EIGHT CHARACTERS OF RYE POLYCROSSES AND THE MULTIPLE CORRELATION COEFFICIENT OF YIELD WITH THE OTHER SEVEN CHARACTERS

Simple		Partial	
Characters associated	Correlation coefficient	Characters associated	Correlation coefficient
AC	+.488**	AC.BDEFGH	+.466**
AG	+.422**	AG.BCDEFH	+.374*
AF	−.373*	AF.BCDEGH	−.237
AB	+.366*	AB.CDEFGH	+.243
AD	+.274	AD.BCEFGH	+.233
AE	+.186	AE.BCDFGH	+.584**
AH	+.017	AH.BCDEFG	+.025
EC	−.283	EC.ABDFGH	−.373*
ED	−.314*	ED.ABCFGH	−.318*
EF	+.402**	EF.ABCDGH	+.349*
	R^2 A.BCDEFG .606		
	R .778**		

Code: A – Yield
B – Fall growth vigour
C – Stooling vigour
D – Date of heading
E – Weight per bu.
F – Pollen sterility
G – Per cent fruitfulness
H – Weight per 100 kernels

Weight per bushel and pollen sterility were highly significantly positively correlated. Thus polycrosses with a high weight per bushel had a slight tendency toward high sterility. Per cent fruitfulness and weight of one hundred kernels were highly significantly positively associated.

Relationship of all characters to at least one of the other characters used in the study was significant at the five per cent or higher level. Therefore, partial correlation coefficients were calculated using these simple correlation coefficients and are presented in Table 4. The appropriate simple correlation coefficients are presented also in this table for purposes of comparison.

The partial correlation coefficient for the association of yield with stooling vigour holding constant the effects of the other characters was highly significant. It is, however, only + 0.466 so that the character, stooling vigour, should be regarded merely as a fair indicator of yield. The association of yield with per cent fruitfulness, eliminating the influence of the other characters, was positively significant at the five per cent level. Per cent fruitfulness was a less reliable criterion of yield than stooling vigour. Both of these associations were closely similar to their respective simple correlation coefficients.

The partial correlation coefficient for the association of yield with per cent pollen sterility approached significance in a negative direction, though the simple correlation for these two characters was significant. The partial correlation coefficient for the association of yield and fall growth vigour and of yield and date of heading both approached significance in a positive direction.

The association of yield with weight per bushel was highly significantly positive when the other six characters were held constant, and was non-significant when these were not held constant. Apparently much of the association between yield and weight per bushel was obscured by the influence of other characters and eliminating this influence brought out the true relationship between yield and weight per bushel.

The only other significant partial correlation coefficients among the eight characters studied also involved the character weight per bushel. Weight per bushel was significantly negatively associated with stooling vigour, when the other six characters were held constant, and also significantly negatively associated with date of heading. The partial correlation coefficient for the association of weight per bushel and pollen sterility was significant positively.

The multiple correlation coefficient, indicating the dependence of yield on the total influence of the other seven characters, is also given in Table 3 and was highly significant. Slightly better than sixty per cent of the squared variability in yield was accounted for by the association with the other seven characters. While this value was highly significant, it still left a great deal of variation in yield unaccounted for by the influence of the characters studied.

The correlations, however, did make available some indication of the relative importance of the characters studied as criteria for the evaluation of the combining ability of the inbreds from their polycross performance.

The relative yield of the various polycrosses in comparison to the standard varieties might be expected to be of primary importance for this purpose. Stooling vigour appeared to be of some importance and, judging by the association with yield, of somewhat more value than per cent fruitfulness. Weight per bushel might be placed next in regard to possible value as an aid in selecting superior inbreds. Fall growth vigour and pollen sterility appeared equally low in value, since each showed about the same low degree of association with yield. Date of heading was probably of slight value and weight per hundred kernels of no value for predicting the possible yield performance of the inbreds in this study. While of no direct value in relation to yield, characters like weight of one hundred kernels and pollen sterility are of some interest in themselves.

Relation of Characters of Inbreds to Polycross Performance

Before making the selections of inbred lines to be recombined as synthetics, it appeared desirable to compare the polycross performance of the inbreds in relation to two characteristics not previously discussed in these studies. These were the length of time of inbreeding and the degree of self-fertility under isolation conditions.

Length of Time of Inbreeding

Data on performance in relation to the length of time of inbreeding are given in Table 5. The weight per hundred kernels appeared to show the effects of selection for plumpness, as there was a generally progressive increase in weight with increased number of years of self-pollination. On the other hand, the four long-time inbred lines had an average relative yield of 102 per cent, while the lines with four or five years' inbreeding had an average yield of 110 per cent; those with two or three years' inbreeding had a yield of 116 per cent, and the twenty-eight lines inbred only one generation, before being tested for combining ability, yielded 119 per cent. From the range in yield for the different groups, it is evident that long-continued self-pollination is unnecessary before testing for yield in polycrosses.

TABLE 5.—POLYCROSS PERFORMANCE OF INBRED SELECTIONS GROUPED ACCORDING TO THE NUMBER OF YEARS OF SELF-POLLINATION

Character	Years self-pollinated			
	1	2 and 3	4 and 5	8, 16 and 19
	Number of inbreds			
	28	6	5	4
Relative yield per cent	119	116	110	102
Range in yield per cent	96–139	104–125	81–131	99–108
Per cent fruitfulness	70	69	67	75
Fall growth vigour	4.1	4.7	4.2	4.0
Stooling vigour	4.3	4.4	3.7	4.0
Weight per bu.—lb.	55.8	56.6	57.4	55.5
Weight per 100 kernels—grams	2.60	2.60	2.80	2.85
Pollen sterility per cent	10.3	11.6	23.0	13.8

Degree of Self-fertility

Classification of the self-pollinated lines for self-fertility was attempted by studying data for the 1945 and 1946 crop seasons. The classification (see Table 6) was made on the basis of the number of plants in each line that were rather highly self-fruitful. Five classes were used: (1) all plants highly self-fertile; (2) most plants self-fertile with an occasional self-unfruitful plant; (3) approximately one-half of the plants highly self-fertile; (4) approximately one-fourth of the plants highly self-fertile; (5) all plants intermediate to low in self-fruitfulness.

In general, the differences in average relative polycross performance were negligible between groups. However, the highly self-fertile group was lowest in yielding ability with a range in relative yield for four lines tested in polycrosses of from 95 per cent to 111 per cent and a mean yield of 103 per cent.

Selection of Inbreds for Recombination as Synthetic Varieties

Having considered the relative value of the characters used in this study as indicators of yield or as of importance in themselves, the next problem is that of employing this information as an aid in selecting inbreds to be recombined as synthetic varieties. It has been mentioned that there were two classes of selections in separate polycrosses, i.e. colourless aleurone and green aleurone. Of those polycrosses tested in performance trials, thirty were progeny of colourless aleurone selections and thirteen were progeny of green selections. In the recombinations, these colour classes were maintained and the selection in the colourless aleurone group is discussed first.

In Table 7, the thirty colourless aleurone selections are listed in the order of their relative polycross yield performance on the basis of the average of the three varieties as 100 per cent. Weight per bushel, weight per hundred kernels, per cent fruitfulness and per cent pollen sterility are given also. Certain polycrosses greatly exceeded the varieties in yield. In selecting for recombination, polycross yield was considered of greatest importance and considerable attention was given to weight per bushel and per cent fruitfulness. Availability of inbred seed was also a factor in selection.

It was decided to combine as many lines as possible that were desirable in all characters making use of as much diversity of origin as was available. Therefore, thirteen inbred lines were combined in this group. These were 2, 3, 10, 25, 34, 37, 41, 50, 52, 55, 59, 61, and 64. These lines appeared

TABLE 6.—RELATIVE YIELDING ABILITY OF POLYCROSSES IN RELATION TO THE DEGREE OF SELF-FERTILITY OF THE INBRED LINES

Classification of inbred lines	No. lines	Range in yield	Mean yield
		per cent	per cent
Highly self-fertile	4	95–111	103.1
Mostly self-fertile	9	81–131	111.3
Half plants self-fertile	12	96–137	117.5
One-quarter plants self-fertile	11	109–139	122.6
Int. to low self-fertility	7	99–132	113.9

TABLE 7.—RELATIVE POLYCROSS PERFORMANCE OF COLOURLESS ALEURONE INBREDS OF RYE

Polycross line number or variety	Relative yield	Weight per bushel	Weight per 100 kernels	Per cent fruitfulness	Per cent pollen sterility
	per cent	lb.	g.		
59	139.4	56.7	2.8	75	10
55	137.2	56.1	2.7	75	10
21	134.9	57.2	2.6	67	15
48	132.7	55.3	2.6	77	10
10	130.6	58.0	2.7	71	20
52	130.0	55.8	2.9	74	10
58	127.9	54.9	2.5	80	5
53	126.1	54.4	2.8	68	5
41	125.9	57.3	2.5	74	15
56	125.7	56.6	2.4	72	5
12	125.1	57.9	2.6	71	15
64	124.6	55.6	2.8	71	10
24	123.6	54.5	2.6	69	5
29	123.2	55.6	2.6	61	10
25	122.8	56.6	2.7	68	10
61	121.4	54.7	2.8	75	10
50	120.7	55.7	2.9	78	20
31	117.2	55.5	2.6	73	5
37	116.6	55.8	2.8	67	10
34	116.0	55.7	2.7	72	15
2	110.8	57.5	3.0	73	15
17	110.6	54.8	2.6	72	10
22	110.5	57.0	2.3	61	15
35	109.2	53.9	2.3	70	10
3	105.1	56.2	3.2	83	15
8	101.0	57.3	2.8	65	20
30	100.2	55.1	2.4	61	10
4	98.8	52.5	2.7	72	10
51	95.7	53.7	2.6	67	10
6	95.4	56.2	2.8	65	20
Dakold	109.7	56.8	2.5	68	—
Emerald	102.5	56.3	2.8	74	—
Imperial	88.1	56.8	2.6	80	—

homozygous for colourless aleurone when the seed colour of the inbred lines was re-examined. The long-time inbred, highly self-fertile line 3 was included because, while it yielded only 5.1 per cent more than the average of the varieties, it excelled in seed size and was otherwise desirable. All other lines selected for this group were, in general, relatively satisfactory in all characters.

It seemed of interest also to combine a smaller number of lines selecting only those that excelled in polycross yield. For this group, six inbred lines were combined. The five highest yielders, lines 59, 55, 21, 48, and 10 with an index of yield performance above 130 per cent were selected. Inbred line 12 which had an index of yield of 125 per cent, and which excelled in weight per bushel was also selected, making three inbreds from Imperial and three from Dakold in this group.

For the green aleurone selections (see Table 8), six inbreds were combined in a group of the better lines and nine inbreds were combined in a second group. All lines were derived from Dakold. The six lines combined in the group of more desirable inbreds, when all characters were considered, were 67, 72, 73, 74, 76, and 78. Inbred 78 in the polycross yielded 107.5

per cent of the average yield of the three varieties, while the remaining lines ranged in yield from 11.6 per cent to 18.6 per cent more than the average of the varieties. The second group selected for combination consisted of the following nine lines: 13, 66, 67, 71, 73, 74, 76, 77, 78, and 81.

A final group of lower yielding lines was selected for recombination to serve as a basis for comparison of the performance of the other combinations. The lines selected for this group included colourless aleurone lines 4, 6, 8, 22, 30, 51 and the green aleurone, low yielding, line 65. Of these lines, 22 and 35 yielded in the polycross about the same as Dakold. The other lines ranged in polycross yield from 88.9 to 101 per cent of the average of the three varieties.

DISCUSSION OF RESULTS

Rye is generally considered to be largely cross-pollinated but, unlike corn, the greater percentage of the plants are rather highly self-sterile. However, the occurrence of more or less highly self-fertile plants is sufficiently frequent that selection within self-fertilized lines might be a reasonable method of breeding, provided, of course, that self-fertility is not, in itself, too undesirable as a plant character. In contrast to some other self-sterile, cross-pollinated plants, rye does not commonly fall into two classes of self-fertility, i.e. highly self-fertile or self-sterile, but commonly shows a wide range in the self-fertility of individual plants of the line in the early generations of self-pollination. Thus it is frequently possible to utilize selection as a means of raising the average self-fertility of a line over a period of years.

Combining highly self-fertile inbreds of rye has been of some value as a means of improving productivity. Such a practice would appear even more effective if lines were combined that had previously been shown to have good combining ability. The polycross method of determining combining ability appears well adapted for use with rye due to its mode of pollination.

Table 8.—Relative polycross performance of green aleurone inbreds of rye

Polycross line number or variety	Relative yield	Weight per bushel	Weight per 100 kernels	Per cent fruitfulness	Per cent pollen sterility
	per cent	lb.	g.		
13	119.6	57.3	2.4	63	10
72	118.6	57.3	2.6	74	10
73	117.9	57.0	2.5	66	15
67	117.6	57.0	2.8	68	10
74	115.9	56.8	2.7	70	15
76	111.6	56.5	2.5	62	15
66	108.2	57.3	2.7	73	20
78	107.5	56.3	2.5	66	10
81	106.8	56.7	2.5	64	5
71	104.4	55.1	2.9	68	10
77	103.1	56.2	2.5	69	10
75	99.3	55.6	2.5	63	5
65	80.9	57.1	2.7	57	50
Emerald	108.9	57.0	2.7	71	—
Dakold	102.4	57.3	2.5	69	—
Imperial	88.9	57.0	2.8	73	—

In this study, the polycross performance of forty-three inbred lines of rye was determined in replicated yield trials for several characters expressing combining ability. For many of these characters, significant differences were found between the polycrosses. However, correlation studies indicated that, of these, only vigour, weight per bushel and per cent of fruitfulness were of value as indicators of high yielding ability in the polycrosses. Weight per hundred kernels and weight per bushel were considered of some importance in themselves. Therefore, yield data, along with these other characters, appeared to be of greatest value in selecting lines to be recombined.

The performance of the polycrosses was studied in relation to two characters of the inbred lines. One of these was the length of time of inbreeding and the results obtained indicated that continued self-pollination and selection was of little value in the production of high yielding polycross progeny. In the material compared, the long-time self-pollinated lines yielded somewhat lower in polycrosses, on the average, than lines self-pollinated for only one generation. Lines self-pollinated for several generations yielded no better in polycrosses than lines self-pollinated for only a single generation. The other inbred character studied was the degree of self-fertility. The inbreds were classified into five groups, according to their self-fertility, ranging from all plants of a line highly self-fertile to all plants relatively low in self-fertility. Only one group differed noticeably in yield. The group classified as highly self-fertile, consisting of four lines that had been self-pollinated three years or more, was somewhat lower in yield, on an average, than the other groups. It appeared, therefore, that homozygosity for high self-fertility was not an advantage in the production of high yielding polycrosses.

These two characters have been studied in alfalfa also. Alfalfa breeding, as practised by several prominent workers, was based to a large extent on selection within inbred lines. Current practice appears more concerned with the isolation of high combining clones without resorting to inbreeding, except when desirable in relation to some particular character. Where combining ability was determined by the polycross method, it has been suggested that high self-fertility was not a desirable character for the clones being combined. High self-fertility may lead to the production of considerable self-pollinated seed in the seed plot, which may produce progeny lacking in hybrid vigour when grown in comparison with cross-pollinated seed.

The polycross has been used in alfalfa breeding as an aid in selecting high combining clones and as a means of combining these selected clones. In a comparison of the polycross method with average top cross and single cross performance, Tysdal and Crandall (5) found that the polycross was as good as either of the other methods as an indicator of the relative combining ability of eight clones. The synthetic variety resulting from recombining clones selected by this method was significantly higher than the standard variety in yield and several other characters.

It appears logical to expect satisfactory results in rye breeding since, in these studies, many of the polycross progenies gave excellent yields in comparison with the standard rye varieties. Apparently there must have been a high degree of cross-pollination in the polycross plots even though the parent plants were selected for high seed production when self-pollinated.

SUMMARY

1. Selection in self-pollinated lines of rye has been practised in a rye improvement study in Minnesota. Individual plants were selected that excelled in seed-setting under isolation, in seed characters and also in vigour. While several lines had been self-pollinated for many years, a considerable proportion had been self-pollinated for only one generation. The majority of the lines were derived from the varieties Dakold and Imperial, though other sources were also represented.

2. Sixty-four colourless aleurone selections of rye that had been self-pollinated one to nineteen generations were grown in an isolated polycross nursery, allowed to interpollinate at random and seed was bulked from all the individual plants of a selection. Twenty-three green aleurone selections, self-pollinated one to eight years, were grown and handled similarly in another isolated polycross nursery.

3. Polycrosses of thirty of the colourless aleurone selections and thirteen of the green selections were selected and grown the following year in replicated yield trials. Group I of the yield trials consisted of twenty-one polycrosses, while Group II consisted of twenty-two polycrosses. The varieties Dakold, Emerald and Imperial were included with each group.

4. Average performance data were reported for each polycross and variety for yield, fall growth vigour, stooling vigour, date of heading, height of plant, weight per bushel, per cent fruitfulness, weight per 100 kernels, and pollen sterility.

5. Analyses of variance indicated that significant differences existed between the polycrosses for each of these characters.

6. In comparison with the standard varieties most of the polycrosses were significantly superior in yield and vigour.

7. Correlation coefficients for the 43 polycrosses as one group were determined for all interrelationships of the characters yield, fall growth vigour, stooling vigour, date of heading, per cent fruitfulness, weight per bushel, pollen sterility, and weight per hundred kernels. Highly significant associations were found between yield with stooling vigour and per cent fruitfulness, between weight per bushel and pollen sterility, between per cent fruitfulness and weight per 100 kernels and negatively between stooling vigour and pollen sterility. Several other associations were significant at the 5 per cent level.

8. Significant partial correlations were found for the association of yield with stooling vigour, weight per bushel, and per cent fruitfulness, eliminating the influence of the other characters in each case.

9. Slightly better than 60 per cent of the squared variability in yield was accounted for by its association with six characters as determined by the multiple correlation.

10. Length of time of inbreeding and degree of self-fertility under isolation were studied in relation to polycross performance. Selection during inbreeding appeared effective in increasing seed size. Selected plants that produced a large number of desirable seeds the first year of self-pollination yielded, in polycrosses, on the average, as well as, or slightly better, than polycrosses from lines self-pollinated from two to five years.

Four inbred lines self-pollinated eight to nineteen years ranged in yield from 99 to 108 per cent of the average of the varieties and were lower in average yield than groups self-pollinated for a shorter period.

The lines studied in polycrosses were placed in five groups for self-fertility as follows: (1) all plants highly self-fertile; (2) most plants self-fertile with an occasional self-unfruitful plant; (3) approximately one-half of the plants highly self-fertile; (4) approximately one-fourth of the plants highly self-fertile; (5) all plants intermediate to low in self-fruitfulness. In general, the groups yielded about the same, although the highly self-fertile group had the lowest average yield.

11. In selecting inbreds for recombination on the basis of combining ability, it appeared that yield of the polycross progeny should be given first consideration. Stooling vigour, per cent fruitfulness, and weight per bushel were of some importance. Other characters though of importance in themselves were not useful as indicators of yield.

12. Five recombinations were set up to test the value of the selections made on the basis of polycross performance.

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STUDIES ON STRONGLY ALKALINE SOILS OF THE SALT RIVER PLAINS IN NORTHWESTERN CANADA[1]

H. J. Atkinson[2], R. F. Bishop[3] and A. Leahey[4]

Dominion Department of Agriculture, Ottawa, Canada

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During the chemical examination of a number of soil samples collected in 1945 from Northwestern Canada, it was observed that certain subsurface samples from the Salt River plains (Figure 1) had extremely high pH values (up to 9.6) but contained only small amounts of water-soluble salts. Such areas supported a very good growth of grass and trees (Figures 2 and 3). On the other hand, certain other samples from barren areas (Figure 4) in the vicinity contained considerably higher concentrations of soluble salts and their reaction did not exceed pH 8.5. It was therefore considered that a more complete study of these soil samples would be of interest.

The area from which the samples were collected lies adjacent to the Salt River about 20 miles southwest of Fort Smith, N.W.T. The river here flows through a level plain of lacustrine and alluvial origin. The area is noted locally for the brine springs which occur at the base of a prominent escarpment marking the western limits of the plain, and for the presence of large flats barren of vegetation.

The four profiles (Table 1) studied in the investigation were selected as being typical of the major mineral soils of the area which form an interesting combination of soils developed under trees, under grass, and without any natural vegetation. Profile D is a saline soil; profile C is an immature chernosem; profile G is a grey wooded soil; profile I is a degraded chernosem.

LABORATORY DETERMINATIONS

In Table 1 are presented the results of certain chemical determinations on the soil samples. These determinations include reaction, nitrogen, calcium carbonate, and content of water-soluble material together with estimated amounts of sodium chloride and sodium carbonate therein. The pH values were determined by means of the glass electrode and nitrogen by a modification of the Kjeldahl method. The CO_2 liberated by treatment with hydrochloric acid was calculated as $CaCO_3$. The water soluble material was obtained by shaking the soil with two and one-half times its weight of water, letting stand over night and passing the extract through a Pasteur-Chamberland filter candle. The filtrates were analysed for calcium, magnesium, potassium, sodium, chloride and sulphate, and the probable amounts of NaCl and Na_2CO_3 were calculated from these results.

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[2] Chemist, Division of Chemistry, Science Service.

[3] Associate Chemist, Division of Chemistry, Science Service.

[4] Soil Specialist, Division of Field Husbandry, Experimental Farms Service.

FIGURE 1.—Map showing Salt River and location of area sampled.

$CaCO_3$ and pH

There appeared to be no relationship between soil reaction and the amount of calcium carbonate present. In profile D, the $CaCO_3$ content ranged from 68 to 27 per cent and the reaction was pH 8.3. In the other profiles where the reaction was pH 8.9 or above, the $CaCO_3$ content did not exceed 20 per cent. Buehrer and Williams (2) in Arizona showed the absence of any correlation between percentage of $CaCO_3$ in soils and their pH values.

Soluble Salts

In profile D where there was little or no vegetation, the water soluble material after ignition averaged over two per cent of the dry soil. At the other locations where there was considerable growth of trees or grass, the amounts of water-soluble constituents were much less. It is worthy of note that the main constituent of the solids extracted from the high salt area was sodium chloride and made up approximately 80 to 90 per cent of the ignited material. Such high concentrations of the chloride ion in Canadian soils is rather unusual. In a study of alkali soils in Saskatchewan,

FIGURE 2.—Area supporting good growth of grass.

Mitchell (7) stated that "the soluble salts present are commonly sulphates of calcium, magnesium and sodium, the chlorides being relatively uncommon." This has been corroborated by other workers (3, 5, 8).

The sodium that could not be accounted for as chlorides and sulphates was calculated as Na_2CO_3. In the extracts from profile D, the sodium that was estimated to be in the carbonate form did not exceed five per cent of the ignited material and the reaction of the soil samples was in every case less than pH 8.5. In profiles C, G and I where samples taken at greater

TABLE 1.—ANALYSIS OF SOIL SAMPLES FROM SELECTED PROFILES

Profile	Vegetation	Depth of sample	N	$CaCO_3$*	pH	Total solids on ignition		
						as per cent of soil	NaCl content	Na_2CO_3 content
		(in.)	%	%			%	%
D	None	0– 6	0.07	67.97	8.3	2.10	89	5
		6–12	0.05	44.78	8.3	1.77	92	3
		12–18	0.04	27.60	8.3	2.48	90	2
C	True upland grassland	0– 6	0.25	—	6.6	0.38	88	7
		6–12	0.14	29.02	8.6	0.63	85	14
		12–18	0.15	19.76	9.3	0.45	—	—
		18–24	0.13	17.59	9.0	0.38	90	13
G	Large poplars	0– 2	1.90	—	6.2	0.09	28	0
		2– 4	0.27	—	5.8	0.06	63	18
		4– 8	0.08	—	6.2	0.03	—	—
		8–12	0.09	1.17	7.7	0.04	—	—
		12–18	0.06	16.76	9.2	0.08	57	47
		18–27	0.05	17.43	9.6	0.10	70	35
I	Prairie	0– 6	0.28	—	6.7	—	—	—
		6–12	0.07	13.93	9.1	0.29	74	22
		12–18	0.06	13.01	8.9	0.23	79	20
		18–24	0.05	9.76	9.0	0.23	77	20

* Calculated from CO_2 liberated by HCl.

TABLE 2.—pH AND EXCHANGEABLE BASES IN THREE PROFILES

(As milliequivalents per 100 gm. air-dry soil)

Profile	Vegetation	Depth of sample	Exchangeable bases				pH
			Ca	Mg	K	Na	
		(in.)					
C	True upland grassland	6–12	15.8	4.4	0.3	18.1	8.6
		12–18	12.4	3.8	0.2	15.7	9.3
		18–24	10.8	2.9	0.2	13.9	9.0
G	Large poplars	4– 8	10.0	3.3	0.2	1.0	6.2
		8–12	18.1	5.4	0.2	1.1	7.7
		12–18	11.6	4.5	0.2	3.7	9.2
		18–27	10.4	4.8	0.1	4.7	9.6
I	Prairie degraded appearance	6–12	9.1	3.6	0.2	10.2	9.1
		12–18	8.7	3.4	0.2	10.5	8.9
		18–24	7.8	3.0	0.1	9.3	9.0

TABLE 3.—EFFECT OF HOT WATER EXTRACTION ON REACTION AND SOLUBLE MATERIAL

Type of extraction	pH of soil		Total solids on ignition		
	Before extraction	After extraction	as per cent of soil	NaCl content	Na_2CO_3 content
				%	%
Profile G					
12 to 18 inches					
Cold water	9.3	9.2	0.16	66	22
Hot water	9.3	8.6	0.47	50	27
Profile I					
18 to 24 inches					
Cold water	9.2	9.6	0.46	68	20
Hot water	9.2	8.6	0.75	54	27

depth (down to 24 and 27 inches) were available, reactions as high as pH 9.6 were found. In these cases, the estimated content of sodium carbonate was usually considerably in excess of ten per cent of the ignited material. Because of the low quantities of soluble salts in these profiles, however, the total amounts of sodium carbonate present would be about the same as in the samples of profile D.

Exchangeable Bases

Samples from profiles C, G and I which had pH values above 8.5 were submitted to more thorough examination. Exchangeable bases were determined by extraction with neutral normal ammonium acetate solution. It was found that large amounts of sodium were present, in most cases exceeding the amounts of exchangeable calcium, as shown in Table 2. Although in profile G, the two samples (12 to 18 inches and 18 to 27 inches) with pH values above 9 had less exchangeable sodium than calcium, the proportion of sodium was much greater, and that of calcium somewhat less, than in the 8 to 12 inch sample whose pH value was 7.7.

FIGURE 3.—Area supporting good growth of trees.

Water Extraction and pH

Samples of these three profiles were also extracted and leached with approximately ten times their weight of cold, distilled water. Although the extracts were found to contain very large amounts of sodium, mostly as chloride but with a considerable quantity as carbonate, the pH values of the soil samples themselves were not reduced to any extent by the extraction. In fact, in some cases, the pH values of the extracted soil were higher than those of the original samples.

In view of the fact that sodium carbonate (Na_2CO_3) is considerably more soluble in hot water (48.5 per cent at 104° C.) than in cold (7.1 per cent at 0° C.) (4), it was decided to determine what effect extraction with hot water would have on the pH values of these soils and on the composition of the extracted material. Accordingly, two samples (profile G, 12 to 18 inches and profile I, 18 to 24 inches) were extracted by placing soil and

TABLE 4.—EFFECT OF LEACHING ON REACTION OF SOILS CONTAINING $CaCO_3$ AND NaCl

Soil	Treatment	pH values	
		Before leaching	After leaching
No. 1	Untreated	7.0	7.4
	$CaCO_3$	7.8	8.5
	$CaCO_3$ + NaCl	7.7	9.1
No. 2	Untreated	8.1	8.6
	NaCl	8.0	9.6

FIGURE 4.—Barren area with salt incrustations.

distilled water in bottles in a hot water bath for several hours, then removing the supernatant liquid and filtering it. This method of extraction was repeated several times, following which the residues were air-dried and their pH values determined. The various successive hot water extracts were combined and analysed. The comparative results of hot and cold water extractions for these samples are given in Table 3. In both cases, extraction with hot water brought about decreases in pH values. Considerably more material was extracted with the hot water and this contained a greater proportion of sodium as the carbonate, less as the chloride.

NaCl, $CaCO_3$ and pH

It was believed that, if the high pH values of these soils were due to the presence of sodium carbonate, this might have been formed by a reaction between calcium carbonate in the soil and sodium chloride from the brine springs which, as mentioned in the introduction, are situated not far from where the samples were taken. In order to determine the effect of NaCl and $CaCO_3$ on soil reaction, a laboratory experiment was conducted on two soils, one of which was neutral in reaction (pH 7.0) whereas the other was strongly alkaline (pH 8.1) and contained much free carbonate as shown by strong effervescence when treated with hydrochloric acid.

The neutral soil (No. 1) was treated with both calcium carbonate and sodium chloride (20 gm. $CaCO_3$ and 4 gm. NaCl to 400 gm. soil). The alkaline soil (No. 2) was treated with sodium chloride only, at the rate used on soil No. 1. These, together with untreated samples, were alternately moistened and dried over a period of six months, at the end of which time, all samples were tested for reaction, then leached with several times their weight of cold, distilled water and again tested for reaction. The results obtained are presented in Table 4.

In soil No. 1, the addition of $CaCO_3$ increased the pH value as expected. In both soils, where NaCl was introduced in the presence of $CaCO_3$, the reaction of the samples before leaching was the same as where NaCl was omitted. In all cases, leaching brought about some increase in pH values. However, the reactions of the NaCl-treated soils were increased to a considerably greater extent and values above pH 9 were obtained.

DISCUSSION

Probably the most useful single determination made on soil samples is that of reaction (9). As mentioned above, Buehrer and Williams (2) showed the absence of any correlation between percentage of $CaCO_3$ in soils and their pH values. Bailey (1) pointed out that pH values higher than 8.5 usually reflect the presence of Na_2CO_3 or other alkaline salt accumulated under poor drainage conditions. McGeorge (6) stated that "among the variables affecting pH are salinity, both concentration and character, and the anions and cations as individuals, the character of the base exchange complex, and the mechanical composition of the soil."

In McGeorge's work, the failure to find any correlation between pH and total salts in the soil was "explained by the observation that HCO_3 and CO_3 ions have opposite effects on pH to the Cl and SO_4 ions . . . . . one should not necessarily expect any relation between total salinity and pH, nor between sodium and pH. The salinity effect on pH appears to be largely due to the anions." McGeorge also observed that "the pH of the soil increases with increase in percentage of replaceable sodium and the ratio of sodium to the total exchange capacity of the soil."

SUMMARY

The results obtained in the investigation of soil samples from the Salt River plains in Northwestern Canada would appear to agree fairly well with those to which reference has just been made. Where the sodium chloride concentration was found to be high (85 to 90 per cent of the total soluble material after ignition) the soil reaction seldom exceeded pH 8.5 although appreciable amounts of sodium carbonate were present. On the other hand, where the soil reaction was in the vicinity of pH 9.0 or higher, the amount of sodium carbonate present was approximately the same as in the samples with lower pH values but the total amount of soluble salts was much less. Thus the ratio of sodium carbonate to the other soluble salts present, rather than the actual amount of sodium carbonate, was indicated to be an important factor in determining the pH value of the soil. In the laboratory experiment where NaCl was mixed with soil in the presence of $CaCO_3$, no change in pH value was obtained until the soil was leached. It would appear, therefore, that the high pH values of the soil samples under investigation were due to the presence of sodium carbonate which could have been formed by the interaction of sodium chloride and calcium carbonate in the soil, followed by leaching.

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A NEW TYPE OF BACTERIAL SPOILAGE IN CANADIAN PROCESS CHEESE[1]

E. G. Hood[2] and J. F. Bowen[3]

Science Service, Ottawa, Canada

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To what extent Canadian manufacturers of process cheese have encountered bacterial spoilage of economic importance is not definitely known. Outbreaks involving two widely separated cheese firms have been drawn to the attention of the Division during the past year. In the first instance reported, the defect was confined to half-pound retail packages of process cheese, while in the second outbreak only the 5-pound process cheese showed spoilage. As the trouble had not been experienced previously in these two plants and had proved to be a costly defect, an explanation was sought as to its cause and method of control.

Griffiths (1) reports instances of spoilage of process cheese by *Clostridium coagulans* which involved a bleaching of the colour, the development of softened and crumbled areas in the cheese, and a fecal odour. In some of the affected areas a small dark slit was distinguishable which was thought to be caused by the formation of gas. The source of the infection was not determined. Other investigators (2) have isolated *Clostridium sporogenes* and *Clostridium welchii* from defective samples of tinned process cheese. In the cases reported, the cheese had undergone a gassy fermentation with the development of a putrid odour. The use of skimmilk powder which contained 2 per cent of lactose was believed to have had a bearing on the development of the defect as the putrefactive condition was observed only in cheese to which skimmilk powder was added. The same types of organisms were isolated from cheese which contained no milk powder and thus no additional lactose, but in no instance did the organisms develop and cause deterioration. The authors conclude that the exact part played by the lactose in its association with the defect was not clearly established. Csiszar (3) found that spores of *Clostridium sporogenes* were destroyed only by temperatures which were too high to make a satisfactory process cheese.

NATURE OF THE DEFECT

The samples of defective process cheese submitted by both manufacturing firms were similar in appearance and of identical odour on examination. In all cases, the cheeses were badly swelled or puffed, contained numerous gas-holes in the centre of the cheese, and were accompanied by a very obnoxious, penetrating, putrefactive odour. Some variation was found in the degree of swelling or bulging, apparently due to the age of the cheese or to retail temperatures of storing and handling, or to the initial amount of the infection. All samples submitted for examination were definitely inedible.

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[1] Contribution No. 290 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

[2] Chief, Dairy Research.

[3] Assistant Bacteriologist.

FIGURE 1. Appearance of the defect in a half-pound package of process cheese.

OCCURRENCE

According to information supplied by the manufacturers, the defect is much more prevalent in summer than in winter and has been found in plant control check samples as well as in the retail trade. While the occurrence of the defect is usually occasional, it has been found in all batches of cheese made on the same day. Control test packages held at 100° F. for observation by the manufacturer showed bulging in 24 to 48 hours, while in other instances 3 to 4 days were required. Comparative test packages held at summer temperature developed the defect more slowly than at 100° F. but eventually showed bulging in 1 to 2 weeks.

PROCESSING

Both manufacturing plants report that standard processing procedures were in use when the trouble was encountered. Temperatures for the effective pasteurization of the cheese from a public health standpoint were used in all cases. Infection from plant equipment was ruled out as a possible source of the cause of the defect. It was, however, revealed that a change had been made in the process cheese mix and that skimmilk powder had been substituted in varying percentages (from 2 to 10) to replace other milk solids. It was also stated by one manufacturer with many years of successful operation that the defect coincided with the introduction of skimmilk powder, and particularly so where high percentages were used. It was also demonstrated by the same manufacturer that the defect did not develop in process cheese with the old formulae which did not call for skimmilk powder, nor did it develop in the half-pound packages containing 2.5 per cent of skimmilk powder.

ISOLATION AND IDENTIFICATION OF ORGANISM

Microscopic examination of the defective cheese showed the presence of large numbers of spores and spore-forming bacteria resembling Clostridia. Tubes of thioglycollate medium + 0.5 per cent starch were inoculated with samples of the cheese and incubated at 37° C. Growth was obtained in 3 days. The cultures were purified by plating on Spray's liver veal agar and by picking colonies into thioglycollate medium. A description of the cultural and biochemical characteristics of the organism follows:

Motile, Gram positive, straight rod with rounded ends, occurring singly, in pairs, or less frequently in short to long chains.

Spores oval, sub-terminal with swelling rods.

Growth on agar slant, spreading, rhizoid and opaque.

Agar colonies woolly with dense centre.

Produces a turbid, flocculent sediment and putrid odour in nutrient broth.

Rapidly digests litmus milk.

Does not blacken brain medium or coagulated albumen. Hydrogen sulphide is produced, but nitrates are not reduced nor indol formed.

Causes fermentation of dextrose and maltose with formation of gas, but does not attack lactose or sucrose.

The organism is anaerobic with an optimum temperature of 37° C

On the basis of these tests, and tests conducted by the Laboratory of Hygiene, Department of National Health and Welfare, the organism was identified as a variant of *Clostridium sporogenes* (4).

MAIN SOURCES OF INFECTION

The main source of the organism causing spoilage has been found to be associated with certain batches of skimmilk powder used in the manufacture of process cheese. While it has not been possible to isolate *Cl. sporogenes* from all samples of skimmilk powder, it has, however, been found in 50 per cent of the samples examined. The organism has also been reported to be present in raw milk Cheddar cheese by one of the commercial plants experiencing trouble. An examination of the sample submitted to our laboratory by this company gave negative results. In the absence of supporting data, it is suspected that some vats of Cheddar cheese may contain the organism. Whereas the source of infection has been definitely traced to skimmilk powder, the main factor in initiating spoilage appears to be related more to its content of available nutrients for the growth of *Cl. sporogenes*.

EXPERIMENTAL

Table 1 shows the effect on experimental batches of process cheese made with the addition of different milk solids when uninoculated and inoculated with spores of *Cl. sporogenes*.

TABLE 1.—EFFECT OF *Cl. sporogenes* ON EXPERIMENTAL PROCESS CHEESE CONTAINING MILK SOLIDS IN THE FORM OF SKIMMILK POWDER, CASEIN DIGEST AND AGED CHEESE

Main ingredients (1)	No. of spores added per gram of process cheese		
	0	1000	5000
4-Months old Cheddar cheese	Normal cheese	Normal cheese	Normal cheese
As above + 2.5 per cent sterile skim powder	Normal cheese	Slight bleaching	+++
As above + 5.0 per cent sterile skim powder	Normal cheese	+ (3)	+++
As above + 10.0 per cent sterile skim powder	Normal cheese	++	+++
As above + 2.0 per cent casein digest (2)	Normal cheese	++	+++
1-Year old Cheddar cheese	Normal cheese	+++	+++

(1) = Experimental batches of process cheese held for 5 days at 98° F.
(2) = N-Z- Case. Sheffield brand of tryptic digested casein.
(3) = + – Definite gas.
++ – Pronounced gas and proteolysis.
+++ – Very pronounced gas and proteolysis.

DISCUSSION

It will be noted in Table 1, column 2, that all of the experimental control batches of process cheese which were free of *Cl. sporogenes*, but which contained different percentages of sterile skimmilk powder and casein

digest, remained normal when incubated at 98° F. for 5 days. Process cheese made from raw Cheddar of 4 months' age but containing no added milk constituents in the form of skimmilk powder or casein digest, remained normal even when inoculated with 5000 *Cl. sporogenes* cells per gram of cheese. Process cheese made to contain 2.5 per cent of sterile skimmilk powder showed only a slight trace of bleaching with no evidence of spoilage when inoculated with 1000 spores per gram of cheese but very marked spoilage with 5000 spores per gram. With the addition of 5.0 and 10.0 per cent of sterile skimmilk powder and 2.0 per cent of casein digest all experimental batches were badly blown and accompanied by a very strong putrefactive odour. Spoilage was more pronounced with the higher inoculum. Process cheese made from one-year old Cheddar cheese but without the addition of skimmilk powder when inoculated showed as marked spoilage as did the process cheese made with the addition of varying percentages of skimmilk powder and the 2.0 per cent casein digest.

It is evident from the data submitted that two factors were involved in spoilage, namely, the load or number of organisms present in the process cheese at the time of manufacture, and secondly, the presence of nutrients which were supplied by the addition of sterile skimmilk powder, casein digest, or from mature Cheddar cheese. The latter factor, however, is much more important than the former, as it has been found impossible to initiate spoilage in highly infected experimental process cheese when no nutrients were added in the form of milk solids. It follows, therefore, that the greater the percentage of skimmilk powder added, the more rapidly will spoilage develop.

While no supporting data are available, it is suspected that some vats of Cheddar cheese may contain the organism in sufficient numbers to cause spoilage if the proper nutrients are provided in the process cheese by the addition of skimmilk powder or by the use of high percentages of mature Cheddar cheese.

PRACTICAL IMPLICATIONS

According to manufacturers, skimmilk powder is an essential and desirable ingredient in the making of process cheese. As this product has been found to be the major source of infection as well as providing nutrients for *Cl. sporogenes* in process cheese, it would appear highly desirable to have all powders used in the manufacture of process cheese examined by special bacteriological methods.

SUMMARY

1. Two widely separated outbreaks of bacterial spoilage in process cheese reported to the Division have been investigated.

2. An organism whose characteristics correspond closely to those of *Clostridium sporogenes* was found to be the causal organism responsible for the defect.

3. The organism has been isolated from defective process cheese and from 50 per cent of the samples of skimmilk powder examined.

4. The defect was not reproduced in experimental batches of process cheese made from four-months-old Cheddar cheese even when heavily inoculated with the organism.

5. The defect was readily reproduced in experimental batches of process cheese made with different percentages of skimmilk powder, with 2.0 per cent casein digest, and with one-year-old Cheddar cheese and inoculated with *Cl. sporogenes*.

6. In addition to being the main source of infection, skimmilk powder furnishes the necessary nutrients for the development of the organism in process cheese.

7. As the number of organisms added to process cheese hastens the development of spoilage, the greater the percentage of infected skimmilk powder used the greater is the amount of infection added.

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THE AMINO ACID COMPOSITION OF THE HORSEBEAN (*VICIA FABA* L.) AS RELATED TO ITS NUTRITIVE VALUE FOR CHICKS[1]

J. H. MAHON[2] AND R. H. COMMON[3]

Macdonald College, Que.

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INTRODUCTION

Canadian crops yielding a supply of protein meal suitable for chickens are relatively few. The horsebean, however, thrives in the lower St. Lawrence area and the Lake St. John area of Quebec Province. Nickolaiczuk, Brisson and Mani (9) have studied the use of horsebeans as a source of vegetable protein for growing chicks. The results showed that the principal amino acid deficiency of horsebean proteins is its low methionine content, and that horsebean protein fortified with this amino acid might be of considerable value as a protein for chicks.

Information on the amino acid composition of horsebean protein appears to be limited. It was decided, therefore, to conduct microbiological assays of its amino acid composition. The results of this study are now presented and discussed in relation to the findings of Nickolaiczuk *et al.* (9).

MATERIAL STUDIED

The sample of horsebeans studied was typical of those grown in Quebec Province. It contained 27.6 per cent crude protein.

PREPARATION OF HYDROLYSATES FOR MICROBIOLOGICAL ASSAY

The whole beans were finely ground and dried to constant weight *in vacuo* at 80° C. Acid hydrolysates were prepared directly from this material by sealing weighed samples in glass tubes with 10 ml. of 10 per cent hydrochloric acid (2.5 N) per gram of meal. The sealed tubes were then autoclaved for 10 hours at 121° C. The cooled hydrolysates were filtered, and any precipitate of humin was broken up and thoroughly washed with distilled water. Some sodium acetate was added and the acidity adjusted to pH 4.0. The hydrolysates were then made up to a suitable volume, covered with a layer of toluene and stored at approximately 5° C. pending assay.

In the case of cystine assays the hydrolysate was prepared from a protein fraction obtained as described below instead of by direct hydrolysis of the ground beans.

Alkaline hydrolysates were used exclusively in the cases of assays for tryptophane and tyrosine. The hydrolysates were prepared in the same manner as the acid hydrolysates, but using 10 ml. 20 per cent sodium hydroxide per gram of material instead of hydrochloric acid.

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[1] Contribution from the Faculty of Agriculture of McGill University, Macdonald College, Que., Canada. Journal Series No. 257.

[2] Graduate student and holder of a scholarship from the Agricultural Institute of Canada,

[3] Professor of Agricultural Chemistry.

EXPERIMENTS ON THE EXTRACTION OF A PROTEIN FRACTION

The large proportion of carbohydrate in the beans leads to heavy losses of cystine if the hydrolysis is carried out directly. Experiments were made, therefore, in order to determine optimal conditions for the extraction of the protein from the ground beans by dilute alkali. Preliminary experiments showed that it was necessary to grind finely in order to obtain satisfactory extraction. Accordingly "fine bean meal" samples were prepared by further grinding the meal for 48 hours at 120 revolutions per minute in a small ball mill. A weighed portion was taken in a centrifuge tube and shaken vigorously for 15 minutes with 50 ml. of 0.08 per cent sodium hydroxide for 15 minutes. The mixture was centrifuged and the clear supernatant was decanted. Two further similar extractions of the residue were carried out. The united clear brown extracts contained 94.3 per cent of the total nitrogen of the fine meal. Further extractions did not appreciably increase the proportion of nitrogen extracted.

A series of similar extractions was carried out using increasing strengths of sodium hydroxide up to 0.40 per cent. Increasing the strength of the alkali resulted in a slightly greater extraction of the total nitrogen, but this trend could not be put to practical use. As the concentration of sodium hydroxide exceeded 0.10 per cent, marked swelling of the residue took place with a tendency to form colloidal suspensions. In order to avoid this difficulty and to secure as complete separation of the protein from the carbohydrate material as possible, it was decided to adhere to the use of 0.08 per cent sodium hydroxide as extractant.

The optimal pH for the precipitation of the protein from the extract was determined by extracting a number of 1.0 gm. samples of fine meal with 0.08 per cent caustic soda as described above. The extracts were collected separately and adjusted to acidities ranging from pH 3.4 to pH 4.4. The resultant precipitates were separated by centrifuging and analysed for nitrogen. The results are set out in Table 1.

TABLE 1.—EFFECT OF pH UPON PRECIPITATION OF PROTEIN FROM ALKALINE EXTRACTS OF HORSEBEAN MEAL

pH	Nitrogen in precipitate expressed as percentage of total nitrogen in sample
4.4	75.9
4.2	76.8
4.0	77.4
3.8	77.7
3.6	77.1
3.4	76.2

The greatest precipitation of protein was secured at pH 3.8. The remaining 22.3 per cent of the total horsebean nitrogen was divided between the extracted residue (5.8 per cent of the total nitrogen) and the supernatant solution (16.5 per cent of the total nitrogen). An amount of the nitrogen in the supernatant corresponding to 12.0 per cent of the total nitrogen of the sample was insoluble in 10 per cent trichloroacetic acid and

was classified as non-protein nitrogen. This figure of 12.0 per cent for the non-protein nitrogen shows that the precipitate obtained by adjusting the alkali extract to pH 3.8 accounted for 88.3 per cent of the protein nitrogen present in the original material.

The optimal conditions for extraction and precipitation of a protein fraction as determined by the foregoing experiments were observed when preparing relatively carbohydrate-free protein precipitates for the cystine assays. Assays of acid hydrolysates of this protein fraction gave almost twice as high a figure for cystine in the whole bean as did assays conducted on direct acid hydrolysates of the meal.

METHODS USED IN MICROBIOLOGICAL ASSAYS

Methionine, leucine, isoleucine and cystine were assayed by Barton-Wright's (2) procedure using *Lactobacillus arabinosus* 17-5; lysine and histidine were assayed by the same procedure, but using *Leuconostoc mesenteroides* P-60. Arginine and threonine were assayed using Barton-Wright's (2) basal medium modified by including double amounts of glucose and sodium acetate; *L. arabinosus* 17-5 was used for the arginine assays and *Streptococcus faecalis* for the threonine assays. Proline, serine and aspartic acid were assayed by the procedure of Sauberlich and Baumann (11) using *L. mesenteroides* P-60. Tyrosine was assayed by the method of Baumgarten *et al.* (4) using *Streptococcus faecalis;* alamine was assayed by the same procedure, but with the modification that the glycine content of the basal medium was reduced from 200 mgm. per litre to 30 mgm. per litre in order to eliminate the initial flat section of the standard response curves. Phenylalanine and valine were assayed according to Horne *et al.* (8) using *L. arabinosus* 17-5, and glycine by the procedure of Dunn *et al.* (6) using *L. mesenteroides* P-60. Tryptophane was assayed by the method of Barton-Wright and Curtis (3), using *L. arabinosus* and extracting the hydrolysate thoroughly with ethyl ether and toluene prior to assay as a precaution against interference from indole and anthranilic acid.

TABLE 2.—AMINO ACID COMPOSITION OF HORSEBEAN (*Vicia faba* L.)

Amino acid	As per cent of whole bean containing 4.42% N	As per cent of horsebean crude protein containing 16% N
Alamine	0.73	2.64
Aspartic acid	1.79	6.46
Arginine	1.64	5.94
Cystine	0.30	1.09
Glutamic acid	3.78	13.68
Glycine	1.03	3.73
Histidine	0.80	2.90
Iso-leucine	1.53	5.54
Leucine	2.10	7.60
Lysine	1.53	5.54
Methionine	0.15	0.53
Phenylalanine	0.95	3.44
Proline	1.25	4.54
Serine	1.58	5.73
Threonine	0.73	2.64
Tryptophane	0.25	0.91
Tryosine	0.85	3.08
Valine	1.42	5.14

Experiments were conducted using increasing amounts of indole and of anthranilic acid instead of tryptophane in order to measure the responsiveness of *L. arabinosus* 17-5 to these analogues. The three substances gave almost identical response curves when supplied in equimolar amounts.

Glutamic acid assays were made using *L. arabinosus* 17-5 and the medium of Hac *et al.* (7) modified by including doubled amounts of glucose and sodium acetate, by using DL-aspartic acid at the rate of 67 mgm. per litre basal medium instead of 400 mgm. L-aspartic acid and by using L-glutamic acid to construct the standard curve.*

DISCUSSION OF ASSAY RESULTS

The complete assay results are summarized in Table 2.

Whole egg protein is known to be of excellent biological value for the chick. When the data for the horsebean are compared with the published data for whole egg proteins (5), as in Table 3, then it is evident that the methionine content of horsebean protein is relatively very low, and that its cystine content is also low. A better comparison from the standpoint of nutritional value for the chick is obtained when the amino acid percentages in the horsebean crude protein are compared with the amino acid percentages calculated for the crude protein of a theoretically adequate ration containing 20 per cent crude protein. The latter figures are based on Almquist's standards (1) for amino acid requirements of the chick, and the comparison is included in Table 3.

The data in Table 3 show that horsebean protein is likely to be very deficient in methionine and cystine if used as the sole source of protein in a chick ration. Glycine, phenylalanine and tryptophane fall below the optimal values given by Almquist (1), but the lysine content is adequate.

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TABLE 3.—COMPARISON OF THE AMINO ACID COMPOSITION OF HORSEBEAN PROTEIN WITH THAT OF (*a*) THE PROTEIN OF WHOLE EGG AND (*b*) OF A PROTEIN THEORETICALLY ADEQUATE FOR THE CHICK AT A LEVEL OF 20 PER CENT CRUDE PROTEIN IN THE DIET

Amino acid	As per cent of horsebean protein containing 16% N	As per cent of whole egg protein containing 16% N	As per cent of theoretically ideal protein for chick nutrition (Almquist (1))
Arginine	6.5	6.4	6.0
Cystine	1.1	2.4	2.0
Glycine	3.7	2.5	5.0
Histidine	2.9	2.1	1.5
Isoleucine	5.5	6.0	3.0
Leucine	7.6	9.2	7.0
Lysine	5.5	7.2	4.5
Methionine	0.5	4.1	2.5
Phenylalanine	3.4	6.3	4.5
Threonine	2.6	4.9	3.0
Tryptophane	0.9	1.5	1.25
Tyrosine	3.1	4.5	—
Valine	5.1	7.3	4.0

TABLE 4.—COMPOSITION OF BASAL RATION USED BY NICKOLAICZUK *et al.* (9)

Constituent	Per cent	Per cent of crude protein contributed by constituent, calculated from av. comp. (10)
Horsebean meal	35	52
Ground wheat	15	12
Hominy feed	20	11
Wheat middlings	10	9
Ground barley	10	7
Alfalfa meal	5	4
Brewers' yeast	2	5
Limestone	2	—
Iodized salt	1	—

Fortified fish oil	100 gm.	per 100 lb. ration
Riboflavin	0.08 gm.	
Manganous sulphate	6 gm.	

The inadequate proportions of phenylalanine and tryptophane do not mean that a marked growth response will follow the feeding of these amino acids along with horsebean protein, for the responses to any amino acid additions are likely to be obscured by the limiting effect of the low methionine content.

The most important aspect of this part of the discussion is the relation of the assay results to the feeling trial results secured by Nikolaiczuk *et al.* (9). These workers used a basal horsebean ration containing 33 per cent ground horsebeans. The composition of this basal horsebean ration is given in Table 4 since it is necessary also to consider the proportions of the total dietary protein supplied by the various ingredients.

Nikolaiczuk *et al.* (9) secured significant increases in rate of body weight gain when this ration was supplemented with DL-methionine, choline or DL-methionine plus choline, or when fish meal replaced part of the horsebean meal to an extent representing 2 per cent crude protein in the total ration.

Additions of tryptophane did not evoke any growth response, but additions of lysine evoked a response which approached statistical significance. In order more fully to interpret the results of Nikolaiczuk *et al.* (9) it is necessary to calculate the over-all amino acid composition of the basal horsebean ration and the basal horsebean ration plus fish meal, and to compare the data with Almquist's (1) standards for amino acid requirements. These calculations have been made using available data for amino acids as given by Block and Bolling (5) and the data for crude protein as given in the Quebec Feeders' Guide (10). The results are set out in Table 5.

The methionine content of horsebean seed and of the basal horsebean ration is very low. This low methionine content was clearly the limiting factor in determining growth on the basal ration. The addition of either methionine or of fish meal raised the methionine content of the ration to

TABLE 5.—COMPARISON OF AMINO ACID COMPOSITION OF HORSEBEAN SEED PROTEIN, OF THE PROTEIN OF THE BASAL HORSEBEAN RATION AND OF THE HORSEBEAN PLUS FISH MEAL RATION WITH THAT OF THE THEORETICALLY ADEQUATE CRUDE PROTEIN BASED ON ALMQUIST'S (1) STANDARDS

Crude protein of	Methionine	Lysine	Phenylalanine	Tryptophane
	%	%	%	%
Horsebean seed	0.5	5.5	3.4	0.9
Basal horsebean ration	1.2	4.0	4.1	1.1
Basal horsebean ration plus fish meal	2.2	4.3	4.3	1.2
Theoretically adequate ration containing 20 per cent crude protein (Almquist (1))	2.5	4.5	4.5	1.25

approximately the value of 2.5 per cent recommended by Almquist (1). This explains the remarkable increase in growth response which Nickolaiczuk *et al.* (9) obtained on adding 1.25 per cent methionine to their basal ration.

From Table 2 it can be seen that horsebean crude protein is adequate in lysine as compared with Almquist's "theoretical" protein. The crude protein of the basal horsebean ration, however, is slightly deficient for optimal growth (see Table 5). This suggests that additions of lysine to the basal horsebean ration would not be likely to give a growth response unless the ration was already supplemented with methionine. Nickolaiczuk *et al.* (9) obtained indications of a response to supplements of lysine, but the effect did not reach the level of statistical significance. The results of the present assays are, therefore, in keeping with the findings of Nickolaiczuk *et al.* (9).

Horsebean seed protein is somewhat deficient in phenylalanine. The basal horsebean ration, however, contains approximately 4.1 per cent phenylalanine against Almquist's (1) recommended optimum of 4.5. An addition of phenylalanine to the basal horsebean ration would have been unlikely to produce any dramatic increase in growth rate.

Horsebean seed protein itself has a rather low tryptophane content compared with Almquist's (1) standard requirement. The basal horsebean ration contains about 90 per cent of Almquist's (1) requirement figure, and the results, therefore, suggest that additions of tryptophane would be unlikely to produce a significant growth response unless the ration was already fortified with methionine. This inference is again in keeping with the experimental results secured by Nikolaiczuk *et al.* (9) where additions of tryptophane to the basal ration did not improve growth.

SUMMARY AND CONCLUSIONS

(a) The results of microbiological assays of the protein of the horsebean (*Vicia faba* L.) for eighteen amino acids are presented.

(b) A simple procedure is described whereby 88 per cent of the true protein of horsebean meal can be extracted with dilute alkali and reprecipitated relatively free from carbohydrate. Cystine assays on acid hydro-

lysates of this fraction gave results approximately half as great again as assays made on direct acid hydrolysates of the meal despite the loss of protein during isolation.

(c) The results are discussed in relation to existing data on the amino deficiencies of the horsebean when used as a source of protein in chick rations. Horsebean protein is relatively very deficient in methionine and cystine, and deficient to a lesser degree in tryptophane, glycine, phenylalanine and threonine. The analytical results confirm the finding of others that the nutritional deficiencies of horsebean meal as a source of protein in chick rations can be largely met by supplementing with methionine. Horsebean protein is likely to be adequate in arginine, histidine, isoleucine, leucine, lysine and valine if used as a sole source of protein for chicks.

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